CERTIFICATE OF EXPRESS MAILING

I hereby certify that this correspondence and provisional patent application are being deposited with the U.S. Postal Service as "EXPRESS MAIL - POST OFFICE TO ADDRESSEE" under 37 CFR 1.10 in an envelope addressed to: Commissioner of Patents and Trademarks, Washington D.C. 20231, on February 10, 1998.

EXPRESS MAIL Mailing Label No. EM198753019

Name of Person mailing Elizabeth Miller

Signature Elizabeth Miller

Date February 10, 1998

Attorney Docket No.
10971464-1

METHODS FOR EVALUATING OLIGONUCLEOTIDE PROBE SEQUENCES

Appendix

This patent application includes an appendix (the "Appendix"), which contains the source code for the software used in carrying out the examples in accordance with the present invention.

A portion of the present disclosure contains material that is subject to copyright protection. The copyright owner has no objection to the facsimile reproduction by anyone of the patent document or the patent disclosure as it appears in the U.S. Patent and Trademark Office patent files or records, but otherwise reserves all copyright rights whatsoever.

BACKGROUND OF THE INVENTION

1. Field of the Invention.

Significant morbidity and mortality are associated with infectious diseases and genetically inherited disorders. More rapid and accurate diagnostic methods are required for better monitoring and treatment of these conditions. Molecular methods using DNA probes, nucleic acid hybridization and *in vitro* amplification techniques are promising methods offering advantages to conventional methods used for patient diagnoses.

Nucleic acid hybridization has been employed for investigating the identity and establishing the presence of nucleic acids. Hybridization is based on

complementary base pairing. When complementary single stranded nucleic acids are incubated together, the complementary base sequences pair to form double-stranded hybrid molecules. The ability of single stranded deoxyribonucleic acid (ssDNA) or ribonucleic acid (RNA) to form a hydrogen bonded structure with a complementary nucleic acid sequence has been employed as an analytical tool in molecular biology research. The availability of radioactive nucleoside triphosphates of high specific activity and the development of methods for their incorporation into DNA and RNA has made it possible to identify, isolate, and characterize various nucleic acid sequences of biological interest. Nucleic acid hybridization has great potential in diagnosing disease states associated with unique nucleic acid sequences. These unique nucleic acid sequences may result from genetic or environmental change in DNA by insertions, deletions, point mutations, or by acquiring foreign DNA or RNA by means of infection by bacteria, molds, fungi, and viruses. The application of nucleic acid hybridization as a diagnostic tool in clinical medicine is limited due to the cost and effort associated with the development of sufficiently sensitive and specific methods for detecting potentially low concentrations of disease-related DNA or RNA present in the complex mixture of nucleic acid sequences found in patient samples.

One method for detecting specific nucleic acid sequences generally involves immobilization of the target nucleic acid on a solid support such as nitrocellulose paper, cellulose paper, diazotized paper, or a nylon membrane. After the target nucleic acid is fixed on the support, the support is contacted with a suitably labeled probe nucleic acid for about two to forty-eight hours. After the above time period, the solid support is washed several times at a controlled temperature to remove unhybridized probe. The support is then dried and the hybridized material is detected by autoradiography or by spectrometric methods. When very low concentrations must be detected, the above method is slow and labor intensive, and nonisotopic labels that are less readily detected than radiolabels are frequently not suitable.

A method for the enzymatic amplification of specific segments of DNA known as the polymerase chain reaction (PCR) method has been described. This *in vitro* amplification procedure is based on repeated cycles of denaturation, oligonucleotide primer annealing, and primer extension by thermophilic

polymerase, resulting in the exponential increase in copies of the region flanked by the primers. The PCR primers, which anneal to opposite strands of the DNA, are positioned so that the polymerase catalyzed extension product of one primer can serve as a template strand for the other, leading to the accumulation of a discrete fragment whose length is defined by the distance between the 5' ends of the oligonucleotide primers.

Other methods for amplifying nucleic acids have also been developed. These methods include single primer amplification, ligase chain reaction (LCR), transcription-mediated amplification methods including 3SR and NASBA, and the Q-beta-replicase method. Regardless of the amplification used, the amplified product must be detected.

One method for detecting nucleic acids is to employ nucleic acid probes that have sequences complementary to sequences in the target nucleic acid. A nucleic acid probe may be, or may be capable of being, labeled with a reporter group or may be, or may be capable of becoming, bound to a support. Detection of signal depends upon the nature of the label or reporter group. Usually, the probe is comprised of natural nucleotides such as ribonucleotides and deoxyribonucleotides and their derivatives although unnatural nucleotide mimetics such as peptide nucleic acids and oligomeric nucleoside phosphonates are also used. Commonly, binding of the probes to the target is detected by means of a label incorporated into the probe. Alternatively, the probe may be unlabeled and the target nucleic acid labeled. Binding can be detected by separating the bound probe or target from the free probe or target and detecting the label. In one approach, a sandwich is formed comprised of one probe, which may be labeled, the target and a probe that is or can become bound to a surface. Alternatively, binding can be detected by a change in the signal-producing properties of the label upon binding, such as a change in the emission efficiency of a fluorescent or chemiluminescent label. This permits detection to be carried out without a separation step. Finally, binding can be detected by labeling the target, allowing the target to hybridize to a surface-bound probe, washing away the unbound target and detecting the labeled target that remains.

Direct detection of labeled target hybridized to surface-bound probes is particularly advantageous if the surface contains a mosaic of different probes that

are individually localized to discrete, known areas of the surface. Such ordered arrays containing a large number of oligonucleotide probes have been developed as tools for high throughput analyses of genotype and gene expression. Oligonucleotides synthesized on a solid support recognize uniquely complementary nucleic acids by hybridization, and arrays can be designed to define specific target sequences, analyze gene expression patterns or identify specific allelic variations. One difficulty in the design of oligonucleotide arrays is that oligonucleotides targeted to different regions of the same gene can show large differences in hybridization efficiency, presumably due, at least in part, to the interplay between the secondary structures of the oligonucleotides and their targets and the stability of the final probe/target hybridization product. A method for predicting which oligonucleotides will show detectable hybridization would substantially decrease the number of iterations required for optimal array design and would be particularly useful when the total number of oligonucleotide probes on the array is limited. A method to predict oligonucleotide hybridization efficiency would also streamline the empirical approaches currently used to select potential antisense therapeutics, which are designed to modulate gene expression *in vivo* by hybridizing to specific messenger RNA (mRNA) molecules and inhibiting their translation into proteins.

While it is well known that the structure of the target nucleic acid affects the affinity of oligonucleotide hybridization, current methods for predicting target structures from the primary sequence fail to predict target regions accessible for oligonucleotide binding. Consequently, selection of oligonucleotides for antisense reagents or oligonucleotide probe arrays has been largely empirical. As most of the target sequence is sequestered by intramolecular base pairing and not accessible for oligonucleotide binding, the process of identifying good oligonucleotides has required large numbers of low efficiency experiments.

The design and implementation of algorithms that effectively predict the ability of oligonucleotides to rapidly and avidly bind to complementary nucleotide sequences has been an important problem in molecular biology since the invention of facile methods for chemical DNA synthesis. The subsequent inventions of the polymerase chain reaction (PCR), antisense inhibition of gene expression and oligonucleotide array methods for performing massively parallel

hybridization experiments have made the need for effective predictive algorithms even more critical.

Previous attempts to solve the nucleic acid probe design problem include PCR primer design software applications (e.g., OLIGO®), neural networks, PCR primer design applications that search for sequences that possess minimal ability to cross-hybridize with other targets present in a sample (e.g., HYBsimulator™), and approaches that attempt to predict the efficiency of antisense sequence suppression of mRNA translation from a combination of predicted nucleic acid duplex melting temperature and predicted target strand structure. The methods that predict effective oligonucleotide primers for performing PCR from DNA templates work well for that application where relatively stringent conditions are employed. This is because PCR experimental design greatly simplifies the prediction problem: hybridization is performed at high temperature, at relatively low ionic strength and in the presence of a large molar excess of oligonucleotide. Under these conditions, the oligonucleotide and target secondary structures are relatively unimportant.

Unfortunately, these conditions do not apply to oligonucleotide arrays, which are usually hybridized under relatively non-denaturing conditions, or to antisense suppression of gene expression, which takes place *in vivo*. Oligonucleotide arrays can contain hundreds of thousands of different sequences and conditions are chosen to allow the oligonucleotide with the lowest melting temperature to hybridize efficiently. These "lowest common denominator" conditions are usually relatively non-denaturing and secondary structure constraints become significant. Accordingly, the above applications require new predictive methods that are capable of estimating the effects of oligonucleotide and target structure on hybridization efficiency. For these reasons, current algorithms for designing PCR primer oligonucleotides fail badly when applied to the problems of oligonucleotide array or antisense oligonucleotide design.

To date, the most effective approach for identifying oligonucleotides with good hybridization efficiency has been an empirical one. Such an approach involves the synthesis of large numbers of oligonucleotide probes for a given target nucleotide sequence. Arrays are formed that include the above oligonucleotide probes. Hybridization experiments are carried out to determine

which of the oligonucleotide probes exhibit good hybridization efficiencies. Examples of such an approach are found in D. Lockhart, et al., Nature Biotech., *infra*, L. Wodicka, et al., Nature Biotechnology, *infra*., and N. Milner et al. Nature Biotech, *infra*. One major drawback to this approach is the vast number of oligonucleotides that must be synthesized in order to achieve a satisfactory result. Typically, about 2%-5% of the test probes synthesized yield acceptable signal levels.

The use of neural networks for oligonucleotide design has also been investigated. Neural networks are easily taught with real data; they therefore afford a general approach to many problems. However, their performance is limited by the "senses" that they are given. An analogy works best here: the human brain is an astoundingly capable neural network, but a blind person cannot be taught to reliably distinguish colors by smell. In addition, a large amount of data is required to adequately teach a neural network to perform its job well. A comprehensive database for either oligonucleotide array design or antisense suppression of gene expression has not been made available. For these reasons, the performance reported to-date of neural network solutions against the probe design problem is mediocre.

Finally, approaches that have attempted to use target nucleic acid folding calculations to predict experimental results inferred to depend upon hybridization efficiency (e.g. antisense suppression of mRNA translation) have so far only demonstrated that the predictions of current nucleic acid folding calculations correlate poorly with observed behavior. The probable reason for this is that the structures predicted by such programs for long sequences are poor predictors of chemical reality; the results of experiments that attempt to confirm the predictions of such calculations support this assessment. Recent improvements to this approach which use predicted RNA structure topology as a predictor of relative RNA/RNA association kinetics have been more successful at forecasting the results of antisense experiments. However, these methods are not computationally efficient, and have so far only been shown to work for targets less than 100 bases long. Such methods are therefore not yet capable of predicting the behavior of full-length mRNA targets, which are typically between 1,000 and 2,000 bases in length.

2. Description of the Related Art.

U.S. Patent No. 5,512,438 (Ecker) discloses the inhibition of RNA expression by forming a pseudo-half knot RNA at the target's RNA secondary structure using antisense oligonucleotides.

Cook, *et al.*, in U.S. Patent No. 5,670,633 discuss sugar-modified oligonucleotides that detect and modulate gene expression.

Antisense oligonucleotide inhibition of the RAS gene is disclosed in U.S. Patent No. 5,582,986 (Monia, *et al.*).

U.S. Patent No. 5,593,834 (Lane, *et al.*) discusses a method of preparing DNA sequences with known ligand binding characteristics.

Mitsuhashi, *et al.*, in U.S. Patent No. 5,556,749 discusses a computerized method for designing optimal DNA probes and an oligonucleotide probe design station.

U.S. Patent No. 5,081,584 (Omichinski, *et al.*) discloses a computer-assisted design of anti-peptides based on the amino acid sequence of a target peptide.

A PCR primer design application that searches for sequences that possess minimal ability to cross-hybridize with other targets present in a sample is available as HYBsimulator™, version 2.0, AGCT, Inc., 2102 Business Center Drive, Suite 170, Irvine, CA 92715 (714) 833-9983.

A PCR primer design software application is available as OLIGO®, version 5.0, National Biosciences, Inc., 3650 Annapolis Lane North, #140, Plymouth, MN 55447 (800) 747-4362.

D. J. Lockhart, *et al.*, Nature Biotech. 14:1675-1684 (1996) describe a neural network approach to the selection of efficient surface-bound oligonucleotide probes.

M. Mitsuhashi, *et al.*, Nature, 367:759-761 (1994) disclose a method for designing specific oligonucleotide probes and primers by modeling the potential cross-hybridization of candidate probes to non-target sequences known to be present in samples.

R. A. Stull, *et al.*, Nuc. Acids Res., 20:3501-3508 (1992) describe a method of predicting the efficacy of antisense oligonucleotides, using predicted target

secondary structure and predicted oligonucleotide/target binding free energy as input parameters.

N. Milner, *et al.*, Nature Biotechnology, 15:537-541 (1997) compare observed patterns of probe hybridization to those expected from the predicted secondary structure of the nucleic acid target.

L. Wodicka, *et al.*, Nature Biotechnology, 15:1359-1367 (1997) describe simple rules for avoiding inefficient and non-specific probes during design and synthesis of oligonucleotides arrays.

J. SantaLucia Jr., *et al.*, Biochemistry, 35:3555 (1996) disclose parameters and methods for the calculation of thermodynamic properties of DNA/DNA homoduplexes.

N. Sugimoto, *et al.*, Biochemistry, 34:11211 (1995) disclose parameters and methods for the calculation of thermodynamic properties of DNA/RNA heteroduplexes.

J.A. Jaeger, *et al.*, Proc. Natl. Acad. Sci. USA, 86:7706 (1989) disclose methods for estimation of the free energy of the most stable intramolecular structure of a single-stranded polynucleotide, by means of a dynamic programming algorithm.

S. F. Altschul, *et al.*, Nature Genetics, 6:119-129 (1994) disclose methods for calculating the complexity and information content of amino acid and nucleic acid sequences.

T. A. Weber and E. Helfand, J. Chem. Phys., 71, 4760 (1979) describe approaches for the modeling of polymer structures by molecular dynamics simulations.

V. Patzel and G. Sczakiel, Nature Biotech., 16, 64-68 (1998) disclose methods for estimating rate constants for association of antisense RNA molecules with mRNA targets by examination of predicted antisense RNA secondary structures.

Light-generated oligonucleotide arrays for rapid DNA sequence analysis is described by A. C. Pease, *et al.*, Proc. Nat. Acad. Sci. USA (1994) 91:5022-5026.

Mitsuhashi discusses basic requirements for designing optimal oligonucleotide probe sequences in J. Clinical Laboratory Analysis (1996) 10:277-284.

Rychlik, *et al.*, discloses a computer program for choosing optimal oligonucleotides for filter hybridization, sequencing and in vitro amplification of DNA in Nucleic Acids Research (1989) 17(21):8543-8551.

A strategy for designing specific antisense oligonucleotide sequences is described by Mitsuhashi in J. Gastroenterol. (1997) 32:282-287.

Mitsuhashi discusses basic requirements for designing optimal PCR primers in J. Clinical Laboratory Analysis (1996) 10:285-293.

Hyndman, *et al.*, disclose software to determine optimal oligonucleotide sequences based on hybridization simulation data in BioTechniques (1996) 20(6):1090-1094.

Eberhardt discloses a shell program for the design of PCR primers using genetics computer group (GCG) software (7.1) on VAX/VMS™ systems in BioTechniques (1992) 13(6):914-917.

Chen, *et al.*, disclose a computer program for calculating the melting temperature of degenerate oligonucleotides used in PCR or hybridization in BioTechniques (1997) 22(6):1158-1160.

Partial thermodynamic parameters for prediction stability and washing behavior of DNA duplexes immobilized on gel matrix is described by Kunitsyn, *et al.*, in J. Biomolecular Structure & Dynamics, ISSN 0739-1102 (1996) 14(1):239-244.

SUMMARY OF THE INVENTION

One embodiment of the present invention is a method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence. A predetermined set of unique oligonucleotide sequences is identified. The unique oligonucleotide sequences are chosen to sample the entire length of a nucleotide sequence that is hybridizable with the target nucleotide sequence. At least one parameter that is predictive of the ability of each of the oligonucleotides specified by the set of sequences to hybridize to the target nucleotide sequence is determined and evaluated for each of the above oligonucleotide sequences. A subset of oligonucleotide sequences within the predetermined set of unique oligonucleotide sequences is identified based on the examination of the parameter values. Finally, oligonucleotide sequences in the subset are identified that are

clustered along one or more regions of the nucleotide sequence that is hybridizable to the target nucleotide sequence. The oligonucleotide probes corresponding to the identified sequences find use in polynucleotide assays particularly where the assays involve oligonucleotide arrays. For a discussion of oligonucleotide arrays, see, e.g., U.S. Patent No. 5,700,637 (E. Southern) and U.S. Patent No. 5,667,667 (E. Southern), the relevant disclosures of which are incorporated herein by reference.

Another embodiment of the present invention is a method for predicting the potential of an oligonucleotide to hybridize to a complementary target nucleotide sequence. A set of overlapping oligonucleotide sequences is identified based on a nucleotide sequence that is complementary to the target nucleotide sequence. At least two parameters that are independently predictive of the ability of each of the oligonucleotides specified by the oligonucleotide sequences to hybridize to the target nucleotide sequence are determined and evaluated for each of the oligonucleotide sequences. Independence is assured by requiring that the parameters be poorly correlated with respect to one another. A subset of oligonucleotide sequences within the set of oligonucleotide sequences is identified based on the examination of the parameter values. Finally, oligonucleotide sequences in the subset are identified that are clustered along one or more regions of the nucleotide sequence that is complementary to the target nucleotide sequence.

Another embodiment of the present invention is a method for predicting the potential of an oligonucleotide to hybridize to a complementary target nucleotide sequence. A set of overlapping oligonucleotide sequences is obtained based on a nucleotide sequence of length L, complementary to the target nucleotide sequence. The oligonucleotide sequences of the set of overlapping oligonucleotide sequences are of identical length N and spaced one nucleotide apart. The set comprises L-N+1 oligonucleotide sequences. Parameters are determined for each of the oligonucleotide sequences of the set of overlapping oligonucleotide sequences. One parameter is the predicted melting temperature of the duplex of each of the oligonucleotides specified by the oligonucleotide sequences and the target nucleotide sequence, corrected for salt concentration. The other parameter is the predicted free energy of the most stable intramolecular

structure of each of the oligonucleotides specified by the oligonucleotide sequences at the temperature of hybridization of the oligonucleotide with the target nucleotide sequence. A subset of oligonucleotide sequences within the set of oligonucleotide sequences is selected based on an examination of the parameter values by establishing cut-off values for each of the parameters. Oligonucleotide sequences in the subset that are clustered along one or more regions of the complementary nucleotide sequence are ranked based on the sizes of the clusters of oligonucleotide sequences. Finally, a subset of the clustered oligonucleotide sequences is selected that statistically samples the clusters of oligonucleotide sequences. The selected sampled subset is used to specify the synthesis of oligonucleotides for experimental evaluation.

Another aspect of the present invention is a computer based method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence. A predetermined number of unique oligonucleotides within a nucleotide sequence that is hybridizable with the target nucleotide sequence is identified under computer control. The oligonucleotides are chosen to sample the entire length of the nucleotide sequence. A value is determined and evaluated under computer control for each of the oligonucleotides for at least one parameter that is independently predictive of the ability of each of the oligonucleotides to hybridize to the target nucleotide sequence. The parameter values are stored. A subset of oligonucleotides within the predetermined number of unique oligonucleotides is identified by examination of the stored parameter values under computer control. Then, oligonucleotides in the subset that are clustered along a region of the nucleotide sequence that is hybridizable to the target nucleotide sequence are identified under computer control.

Another aspect of the present invention is a computer system for conducting a method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence. The system comprises (a) input means for introducing a target nucleotide sequence into the computer system, (b) means for determining a number of unique oligonucleotide sequences that are within a nucleotide sequence that is hybridizable with the target nucleotide sequence where the oligonucleotide sequences are chosen to sample the entire length of the nucleotide sequence, (c) memory means for storing the oligonucleotide

sequences, (d) means for controlling the computer system to carry out for each of the oligonucleotide sequences a determination and evaluation of a value for at least one parameter that is independently predictive of the ability of each of the oligonucleotide sequences to hybridize to the target nucleotide sequence, (e) means for storing the parameter values, (f) means for controlling the computer to carry out an identification from the stored parameter values a subset of oligonucleotide sequences within the number of unique oligonucleotide sequences based on the examination of the parameter, (g) means for storing the subset of oligonucleotides, (h) means for controlling the computer to carry out an identification of oligonucleotide sequences in the subset that are clustered along a region of the nucleotide sequence that is hybridizable to the target nucleotide sequence, (i) means for storing the oligonucleotide sequences in the subset, and (j) means for outputting data relating to the oligonucleotide sequences in the subset.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig.1 is a general flow chart depicting the method of the present invention.

Fig. 2 is a flow chart depicting a preferred embodiment of a method in accordance with the present invention.

Fig. 3 is a contour plot of normalized hybridization intensity from multiple experiments, as a function of the free energy of the most stable probe intramolecular structure (ΔG_{MFOLD}) and the difference between the predicted RNA/DNA heteroduplex melting temperature (T_m) and the temperature of hybridization (T_{hyb}).

Fig. 4 shows the observed hybridization patterns for oligonucleotides selected using a method in accordance with the present invention and additional oligonucleotides to a portion of the rabbit β-globin gene (radiolabeled antisense RNA target).

Fig. 5 shows the observed hybridization patterns for oligonucleotides selected using a method in accordance with the present invention and additional oligonucleotides to the HIV PRT gene (fluorescein-labeled sense RNA target).

Fig. 6 shows the observed hybridization patterns for oligonucleotides selected using a method in accordance with the present invention and additional oligonucleotides to the G3PDH gene (fluorescein-labeled antisense RNA target).

Fig. 7 shows the observed hybridization patterns for oligonucleotides selected using a method in accordance with the present invention and additional oligonucleotides to the p53 gene (fluorescein-labeled antisense RNA target).

Fig. 8 shows the observed hybridization patterns for oligonucleotides selected using a method in accordance with the present invention and additional oligonucleotides to the HIV PRTs gene (using data from the GeneChip™ data).

DEFINITIONS

Before proceeding further with a description of the specific embodiments of the present invention, a number of terms will be defined.

Nucleic Acids:

Polynucleotide -- a compound or composition that is a polymeric nucleotide or nucleic acid polymer. The polynucleotide may be a natural compound or a synthetic compound. In the context of an assay, the polynucleotide is often referred to as a polynucleotide analyte. The polynucleotide can have from about 20 to 5,000,000 or more nucleotides. The larger polynucleotides are generally found in the natural state. In an isolated state the polynucleotide can have about 30 to 50,000 or more nucleotides, usually about 100 to 20,000 nucleotides, more frequently 500 to 10,000 nucleotides. It is thus obvious that isolation of a polynucleotide from the natural state often results in fragmentation. The polynucleotides include nucleic acids, and fragments thereof, from any source in purified or unpurified form including DNA (dsDNA and ssDNA) and RNA, including tRNA, mRNA, rRNA, mitochondrial DNA and RNA, chloroplast DNA and RNA, DNA/RNA hybrids, or mixtures thereof, genes, chromosomes, plasmids, the genomes of biological material such as microorganisms, e.g., bacteria, yeasts, viruses, viroids, molds, fungi, plants, animals, humans, and the like. The polynucleotide can be only a minor fraction of a complex mixture such as a biological sample. Also included are genes, such as hemoglobin gene for sickle-cell anemia, cystic fibrosis gene, oncogenes, cDNA, and the like.

The polynucleotide can be obtained from various biological materials by procedures well known in the art. The polynucleotide, where appropriate, may be cleaved to obtain a fragment that contains a target nucleotide sequence, for example, by shearing or by treatment with a restriction endonuclease or other site specific chemical cleavage method.

For purposes of this invention, the polynucleotide, or a cleaved fragment obtained from the polynucleotide, will usually be at least partially denatured or single stranded or treated to render it denatured or single stranded. Such treatments are well known in the art and include, for instance, heat or alkali treatment, or enzymatic digestion of one strand. For example, dsDNA can be heated at 90-100° C. for a period of about 1 to 10 minutes to produce denatured material.

Target nucleotide sequence -- a sequence of nucleotides to be identified, usually existing within a portion or all of a polynucleotide, usually a polynucleotide analyte. The identity of the target nucleotide sequence generally is known to an extent sufficient to allow preparation of various sequences hybridizable with the target nucleotide sequence and of oligonucleotides, such as probes and primers, and other molecules necessary for conducting methods in accordance with the present invention, an amplification of the target polynucleotide, and so forth.

The target sequence usually contains from about 30 to 5,000 or more nucleotides, preferably 50 to 1,000 nucleotides. The target nucleotide sequence is generally a fraction of a larger molecule or it may be substantially the entire molecule such as a polynucleotide as described above. The minimum number of nucleotides in the target nucleotide sequence is selected to assure that the presence of a target polynucleotide in a sample is a specific indicator of the presence of polynucleotide in a sample. The maximum number of nucleotides in the target nucleotide sequence is normally governed by several factors: the length of the polynucleotide from which it is derived, the tendency of such polynucleotide to be broken by shearing or other processes during isolation, the efficiency of any procedures required to prepare the sample for analysis (e.g. transcription of a DNA template into RNA) and the efficiency of detection and/or amplification of the target nucleotide sequence, where appropriate.

Oligonucleotide -- a polynucleotide, usually single stranded, usually a synthetic polynucleotide but may be a naturally occurring polynucleotide. The oligonucleotide(s) are usually comprised of a sequence of at least 5 nucleotides, preferably, 10 to 100 nucleotides, more preferably, 20 to 50 nucleotides, and usually 10 to 30 nucleotides, more preferably, 20 to 30 nucleotides, and desirably about 25 nucleotides in length.

Various techniques can be employed for preparing an oligonucleotide. Such oligonucleotides can be obtained by biological synthesis or by chemical synthesis. For short sequences (up to about 100 nucleotides), chemical synthesis will frequently be more economical as compared to the biological synthesis. In addition to economy, chemical synthesis provides a convenient way of incorporating low molecular weight compounds and/or modified bases during specific synthesis steps. Furthermore, chemical synthesis is very flexible in the choice of length and region of the target polynucleotide binding sequence. The oligonucleotide can be synthesized by standard methods such as those used in commercial automated nucleic acid synthesizers. Chemical synthesis of DNA on a suitably modified glass or resin can result in DNA covalently attached to the surface. This may offer advantages in washing and sample handling. For longer sequences standard replication methods employed in molecular biology can be used such as the use of M13 for single stranded DNA as described by J. Messing (1983) Methods Enzymol, 101:20-78.

Other methods of oligonucleotide synthesis include phosphotriester and phosphodiester methods (Narang, *et al.* (1979) Meth. Enzymol 68:90) and synthesis on a support (Beaucage, *et al.* (1981) Tetrahedron Letters 22:1859-1862) as well as phosphoramidite techniques (Caruthers, M. H., *et al.*, "Methods in Enzymology," Vol. 154, pp. 287-314 (1988)) and others described in "Synthesis and Applications of DNA and RNA," S.A. Narang, editor, Academic Press, New York, 1987, and the references contained therein. The chemical synthesis via a photolithographic method of spatially addressable arrays of oligonucleotides bound to glass surfaces is described by A. C. Pease, *et al.*, Proc. Nat. Acad. Sci. USA (1994) 91:5022-5026.

Oligonucleotide probe -- an oligonucleotide employed to bind to a portion of a polynucleotide such as another oligonucleotide or a target nucleotide sequence.

The design and preparation of the oligonucleotide probes are generally dependent upon the sensitivity and specificity required, the sequence of the target polynucleotide and, in certain cases, the biological significance of certain portions of the target polynucleotide sequence.

Oligonucleotide primer(s) -- an oligonucleotide that is usually employed in a chain extension on a polynucleotide template such as in, for example, an amplification of a nucleic acid. The oligonucleotide primer is usually a synthetic nucleotide that is single stranded, containing a sequence at its 3'-end that is capable of hybridizing with a defined sequence of the target polynucleotide. Normally, an oligonucleotide primer has at least 80%, preferably 90%, more preferably 95%, most preferably 100%, complementarity to a defined sequence or primer binding site. The number of nucleotides in the hybridizable sequence of an oligonucleotide primer should be such that stringency conditions used to hybridize the oligonucleotide primer will prevent excessive random non-specific hybridization. Usually, the number of nucleotides in the oligonucleotide primer will be at least as great as the defined sequence of the target polynucleotide, namely, at least ten nucleotides, preferably at least 15 nucleotides, and generally from about 10 to 200, preferably 20 to 50, nucleotides.

In general, in primer extension, amplification primers hybridize to, and are extended along (chain extended), at least the target nucleotide sequence within the target polynucleotide and, thus, the target sequence acts as a template. The extended primers are chain "extension products." The target sequence usually lies between two defined sequences but need not. In general, the primers hybridize with the defined sequences or with at least a portion of such target polynucleotide, usually at least a ten-nucleotide segment at the 3'-end thereof and preferably at least 15, frequently a 20 to 50 nucleotide segment thereof.

Nucleoside triphosphates -- nucleosides having a 5'-triphosphate substituent. The nucleosides are pentose sugar derivatives of nitrogenous bases of either purine or pyrimidine derivation, covalently bonded to the 1'-carbon of the pentose sugar, which is usually a deoxyribose or a ribose. The purine bases include adenine (A), guanine (G), inosine (I), and derivatives and analogs thereof. The pyrimidine bases include cytosine (C), thymine (T), uracil (U), and derivatives and analogs thereof. Nucleoside triphosphates include deoxyribonucleoside

triphosphates such as the four common deoxyribonucleoside triphosphates dATP, dCTP, dGTP and dTTP and ribonucleoside triphosphates such as the four common triphosphates rATP, rCTP, rGTP and rUTP.

The term "nucleoside triphosphates" also includes derivatives and analogs thereof, which are exemplified by those derivatives that are recognized and polymerized in a similar manner to the underivatized nucleoside triphosphates.

Nucleotide -- a base-sugar-phosphate combination that is the monomeric unit of nucleic acid polymers, i.e., DNA and RNA. The term "nucleotide" as used herein includes modified nucleotides as defined below.

DNA -- deoxyribonucleic acid.

RNA -- ribonucleic acid.

Modified nucleotide -- a unit in a nucleic acid polymer that contains a modified base, sugar or phosphate group. The modified nucleotide can be produced by a chemical modification of the nucleotide either as part of the nucleic acid polymer or prior to the incorporation of the modified nucleotide into the nucleic acid polymer. For example, the methods mentioned above for the synthesis of an oligonucleotide may be employed. In another approach a modified nucleotide can be produced by incorporating a modified nucleoside triphosphate into the polymer chain during an amplification reaction. Examples of modified nucleotides, by way of illustration and not limitation, include dideoxynucleotides, derivatives or analogs that are biotinylated, amine modified, alkylated, fluorophore-labeled, and the like and also include phosphorothioate, phosphite, ring atom modified derivatives, and so forth.

Nucleoside -- is a base-sugar combination or a nucleotide lacking a phosphate moiety.

Nucleotide polymerase -- a catalyst, usually an enzyme, for forming an extension of a polynucleotide along a DNA or RNA template where the extension is complementary thereto. The nucleotide polymerase is a template dependent polynucleotide polymerase and utilizes nucleoside triphosphates as building blocks for extending the 3'-end of a polynucleotide to provide a sequence complementary with the polynucleotide template. Usually, the catalysts are enzymes, such as DNA polymerases, for example, prokaryotic DNA polymerase (I, II, or III), T4 DNA polymerase, T7 DNA polymerase, Klenow fragment, reverse

transcriptase, Vent DNA polymerase, Pfu DNA polymerase, Taq DNA polymerase, and the like, or RNA polymerases, such as T3 and T7 RNA polymerases. Polymerase enzymes may be derived from any source such as cells, bacteria such as E. coli, plants, animals, virus, thermophilic bacteria, and so forth.

Amplification of nucleic acids or polynucleotides -- any method that results in the formation of one or more copies of a nucleic acid or polynucleotide molecule (exponential amplification) or in the formation of one or more copies of only the complement of a nucleic acid or polynucleotide molecule (linear amplification).

Hybridization (hybridizing) and binding -- in the context of nucleotide sequences these terms are used interchangeably herein. The ability of two nucleotide sequences to hybridize with each other is based on the degree of complementarity of the two nucleotide sequences, which in turn is based on the fraction of matched complementary nucleotide pairs. The more nucleotides in a given sequence that are complementary to another sequence, the more stringent the conditions can be for hybridization and the more specific will be the binding of the two sequences. Increased stringency is achieved by elevating the temperature, increasing the ratio of co-solvents, lowering the salt concentration, and the like.

Hybridization efficiency -- the productivity of a hybridization reaction, measured as either the absolute or relative yield of oligonucleotide probe/polynucleotide target duplex formed under a given set of conditions in a given amount of time.

Homologous or substantially identical polynucleotides -- In general, two polynucleotide sequences that are identical or can each hybridize to the same polynucleotide sequence are homologous. The two sequences are homologous or substantially identical where the sequences each have at least 90%, preferably 100%, of the same or analogous base sequence where thymine (T) and uracil (U) are considered the same. Thus, the ribonucleotides A, U, C and G are taken as analogous to the deoxynucleotides dA, dT, dC, and dG, respectively. Homologous sequences can both be DNA or one can be DNA and the other RNA.

Complementary -- Two sequences are complementary when the sequence of one can bind to the sequence of the other in an anti-parallel sense wherein the 3'-end of each sequence binds to the 5'-end of the other sequence and each A, T(U), G, and C of one sequence is then aligned with a T(U), A, C, and G, respectively, of the other sequence. RNA sequences can also include complementary G/U or U/G basepairs.

Member of a specific binding pair ("sbp member") -- one of two different molecules, having an area on the surface or in a cavity that specifically binds to and is thereby defined as complementary with a particular spatial and polar organization of the other molecule. The members of the specific binding pair are referred to as cognates or as ligand and receptor (antiligand). These may be members of an immunological pair such as antigen-antibody, or may be operator-repressor, nuclease-nucleotide, biotin-avidin, hormones-hormone receptors, nucleic acid duplexes, IgG-protein A, DNA-DNA, DNA-RNA, and the like.

Ligand -- any compound for which a receptor naturally exists or can be prepared.

Receptor ("antiligand") -- any compound or composition capable of recognizing a particular spatial and polar organization of a molecule, e.g., epitopic or determinant site. Illustrative receptors include naturally occurring receptors, e.g., thyroxine binding globulin, antibodies, enzymes, Fab fragments, lectins, nucleic acids, repressors, protection enzymes, protein A, complement component C1q, DNA binding proteins or ligands and the like.

Oligonucleotide Properties:

Potential of an oligonucleotide to hybridize -- the combination of duplex formation rate and duplex dissociation rate that determines the amount of duplex nucleic acid hybrid that will form under a given set of experimental conditions in a given amount of time.

Parameter -- a factor that provides information about the hybridization of an oligonucleotide with a target nucleotide sequence. Generally, the factor is one that is predictive of the ability of an oligonucleotide to hybridize with a target

nucleotide sequence. Such factors include composition factors, thermodynamic factors, chemosynthetic efficiencies, kinetic factors, and the like.

Parameter predictive of the ability to hybridize -- a parameter calculated from a set of oligonucleotide sequences wherein the parameter positively correlates with observed hybridization efficiencies of those sequences. The parameter is, therefore, predictive of the ability of those sequences to hybridize. "Positive correlation" can be rigorously defined in statistical terms. The correlation coefficient $\rho_{x,y}$ of two experimentally measured discreet quantities x and y (N values in each set) is defined as

$$\rho_{x,y} = \frac{\text{Covariance}(x,y)}{\sqrt{\text{Variance}(x)\text{Variance}(y)}},$$

where the Covariance (x,y) is defined by

$$\text{Covariance}(x,y) = \frac{1}{N}\sum_{j=1}^{N}(x_j - \mu_x)(y_j - \mu_y).$$

The quantities μ_x and μ_y are the averages of the quantities x and y, while the variances are simply the squares of the standard deviations (defined below). The correlation coefficient is a dimensionless (unitless) quantity between –1 and 1. A correlation coefficient of 1 or –1 indicates that x and y have a linear relationship with a positive or negative slope, respectively. A correlation coefficient of zero indicates no relationship; for example, two sets of random numbers will yield a correlation coefficient near zero. Intermediate correlation coefficients indicate intermediate degrees of relatedness between two sets of numbers. The correlation coefficient is a good statistical measure of the degree to which one set of numbers predicts a second set of numbers.

Composition factor -- a numerical factor based solely on the composition or sequence of an oligonucleotide without involving additional parameters, such as experimentally measured nearest-neighbor thermodynamic parameters. For instance, the fraction (G+C), given by the formula

$$f_{GC} = \frac{n_G + n_C}{n_G + n_C + n_A + n_{T\,or\,U}},$$

where n_G, n_C, n_A and $n_{T\,or\,U}$ are the numbers of G, C, A and T (or U) bases in an oligonucleotide, is an example of a composition factor. Examples of composition factors, by way of illustration and not limitation, are mole fraction (G+C), percent (G+C), sequence complexity, sequence information content, frequency of occurrence of specific oligonucleotide sequences in a sequence database and so forth.

Thermodynamic factor -- numerical factors that predict the behavior of an oligonucleotide in some process that has reached equilibrium. For instance, the free energy of duplex formation between an oligonucleotide and its complement is a thermodynamic factor. Thermodynamic factors for systems that can be subdivided into constituent parts are often estimated by summing contributions from the constituent parts. Such an approach is used to calculate the thermodynamic properties of oligonucleotides.

Examples of thermodynamic factors, by way of illustration and not limitation, are predicted duplex melting temperature, predicted enthalpy of duplex formation, predicted entropy of duplex formation, free energy of duplex formation, predicted melting temperature of the most stable intramolecular structure of the oligonucleotide or its complement, predicted enthalpy of the most stable intramolecular structure of the oligonucleotide or its complement, predicted entropy of the most stable intramolecular structure of the oligonucleotide or its complement, predicted free energy of the most stable intramolecular structure of the oligonucleotide or its complement, predicted melting temperature of the most stable hairpin structure of the oligonucleotide or its complement, predicted enthalpy of the most stable hairpin structure of the oligonucleotide or its complement, predicted entropy of the most stable hairpin structure of the oligonucleotide or its complement, predicted free energy of the most stable hairpin structure of the oligonucleotide or its complement, thermodynamic partition function for intramolecular structure of the oligonucleotide or its complement and the like.

Chemosynthetic efficiency -- oligonucleotides and nucleotide sequences may both be made by sequential polymerization of the constituent nucleotides. However, the individual addition steps are not perfect; they instead proceed with some fractional efficiency that is less than unity. This may vary as a function of position in the sequence. Therefore, what is really produced is a family of molecules that consists of the desired molecule plus many truncated sequences. These "failure sequences" affect the observed efficiency of hybridization between an oligonucleotide and its complementary target. Examples of chemosynthetic efficiency factors, by way of illustration and not limitation, are coupling efficiencies, overall efficiencies of the synthesis of a target nucleotide sequence or an oligonucleotide probe, and so forth.

Kinetic factor -- numerical factors that predict the rate at which an oligonucleotide hybridizes to its complementary sequence or the rate at which the hybridized sequence dissociates from its complement are called kinetic factors. Examples of kinetic factors are steric factors calculated via molecular modeling or measured experimentally, rate constants calculated via molecular dynamics simulations, associative rate constants, dissociative rate constants, enthalpies of activation, entropies of activation, free energies of activation, and the like.

Predicted duplex melting temperature -- the temperature at which an oligonucleotide mixed with a hybridizable nucleotide sequence is predicted to form a duplex structure (double-helix hybrid) with 50% of the hybridizable sequence. At higher temperatures, the amount of duplex is less than 50%; at lower temperatures, the amount of duplex is greater than 50%. The melting temperature T_m (°C) is calculated from the enthalpy (ΔH), entropy (ΔS) and C, the concentration of the most abundant duplex component (for hybridization arrays, the soluble hybridization target), using the equation

$$T_m = \frac{\Delta H}{\Delta S + R \ln C} - 273.15,$$

where R is the gas constant, 1.987 cal/(mole-°K). For longer sequences (>100 nucleotides), T_m can also be estimated from the mole fraction (G+C), χ_{G+C}, using the equation

$T_m = 81.5 + 41.0\,\chi_{G+C}$.

Melting temperature corrected for salt concentration -- polynucleotide duplex melting temperatures are calculated with the assumption that the concentration of sodium ion, Na^+, is 1 M. Melting temperatures T'_m calculated for duplexes formed at different salt concentrations are corrected via the semi-empirical equation

$$T'_m\left([Na^+]\right) = T_m + 16.6\,\log\left([Na^+]\right) .$$

Predicted enthalpy, entropy and free energy of duplex formation -- the enthalpy (ΔH), entropy and free energy (ΔG) are thermodynamic state functions, related by the equation

$$\Delta G = \Delta H - T\,\Delta S ,$$

where T is the temperature in °K. In practice, the enthalpy and entropy are predicted via a thermodynamic model of duplex formation (the "nearest neighbor" model which is explained in more detail below), and used to calculate the free energy and melting temperature.

Predicted free energy of the most stable intramolecular structure of an oligonucleotide or its complement -- single-stranded DNA and RNA molecules that contain self-complementary sequences can form intramolecular secondary structures. For instance, the oligonucleotide

5′-ACTGGCAATCACAATTGCCAGTAA-3′ (SEQ ID NO:1)

can base pair with itself, to form the structure

```
  5′-ACTGGCAATCA
     |||||||||| C      (SEQ ID NO:1)
3′-AATGACCGTTAA
```

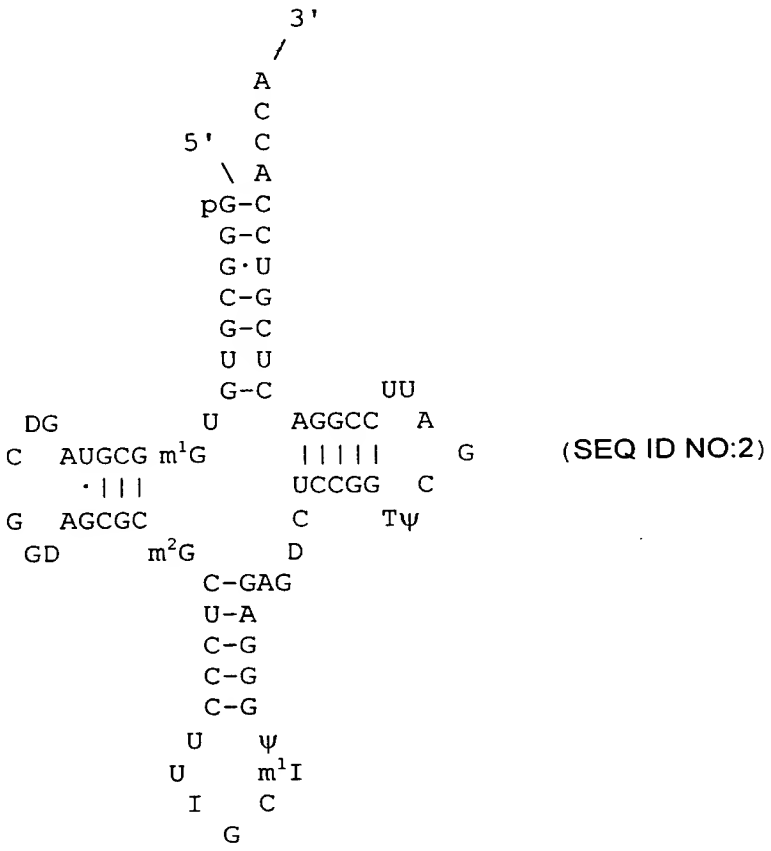
where a vertical line indicates Watson-Crick base pair formation. Many such structures are possible for a given sequence; two are of particular interest. The first is the lowest energy "hairpin" structure (formed by folding a sequence back on itself with a connecting loop at least 3 nucleotides long). The second is the lowest energy structure that can be formed by including more complex topologies, such as "bulge loops" (unpaired duplexes between two regions of base-paired duplex) and cloverleaf structures, where 3 base-paired stretches meet at a triple-junction. A good example of a complex secondary structure is the structure of a tRNA molecule, an example of which, namely, yeast $tRNA^{Ala}$ is shown below.

For either type of structure, a value of the free energy of that structure can be calculated, relative to the unpaired strand, by means of a thermodynamic model similar to that used to calculate the free energy of a base-paired duplex structure. Again, the free energy ΔG is calculated from the enthalpy ΔH and the entropy ΔS at a given absolute temperature T via the equation

$$\Delta G = \Delta H - T\,\Delta S\,.$$

However, in this case there is the added difficulty that the lowest energy structure must be found. For a simple hairpin structure, this optimization can be performed via a relatively simple search algorithm. For more complex structures (such as a cloverleaf) a dynamic programming algorithm, such as that implemented in the program MFOLD, must be used.

Yeast $tRNA^{Ala}$ - The RNA sequence includes many non-standard ribonucleotides, such as D (5,6 dihydrouridine), m^1G (1-methylguanosine), m^2G (N^2-dimethylguanosine), ψ (pseudouridine), I (inosine), m^1I (1-methylinosine) and T (ribothymidine). Dots (·) mark (non-standard) G=U base pairs. The structure is taken from A. L. Lehninger, *et al.*, Principles of Biochemistry, 2nd Ed. (Worth Publishers, New York, NY, 1993).



(SEQ ID NO:2)

Coupling efficiencies -- chemosynthetic efficiencies are called coupling efficiencies when the synthetic scheme involves successive attachment of different monomers to a growing oligomer; a good example is oligonucleotide synthesis via phosphoramidite coupling chemistry.

Algorithmic Operations:

Evaluating a parameter -- determination of the numerical value of a numerical descriptor of a property of an oligonucleotide sequence by means of a formula, algorithm or look-up table.

Filter -- a mathematical rule or formula that divides a set of numbers into two subsets. Generally, one subset is retained for further analysis while the other is discarded. If the division into two subsets is achieved by testing the numbers against a simple inequality, then the filter is referred to as a "cut-off". In the context of the current invention, an example by way of illustration and not limitation is the statement "The predicted self structure free energy must be

greater than or equal to –0.4 kcal/mole," which can be used as a filter for oligonucleotide sequences; this particular filter is also an example of a cut-off.

Filter set -- A set of rules or formulae that successively winnow a set of numbers by identifying and discarding subsets that do not meet specific criteria. In the context of the current invention, an example by way of illustration and not limitation is the compound statement "the predicted self structure free energy must be greater than or equal to –0.4 kcal/mole and the predicted RNA/DNA heteroduplex melting temperature must lie between 60°C and 85°C," which can be used as a filter set for oligonucleotide sequences.

Examining a parameter -- comparing the numerical value of a parameter to some cutoff-value or filter.

Statistical sampling of a cluster -- extraction of a subset of oligonucleotides from a cluster of oligonucleotides based upon some statistical measure, such as rank by oligonucleotide starting position in the sequence complementary to the target sequence.

First quartile, median and third quartile -- If a set of numbers is ranked by value, then the value that divides the lower ¼ from the upper ¾ of the set is the first quartile, the value that divides the set in half is the median and the value that divides the lower ¾ from the upper ¼ of the set is the third quartile.

Poorly correlated -- If it is not possible to perform a "good" prediction, as defined via statistics, of one set of numbers from another set of numbers using a simple linear model, then the two sets of numbers are said to be poorly correlated.

Computer program -- a written set of instructions that symbolically instructs an appropriately configured computer to execute an algorithm that will yield desired outputs from some set of inputs. The instructions may be written in one or several standard programming languages, such as C, C++, Visual BASIC, FORTRAN or the like. Alternatively, the instructions may be written by imposing a template onto a general-purpose numerical analysis program, such as a spreadsheet.

Experimental System Components:

Small organic molecule -- a compound of molecular weight less than 1500, preferably 100 to 1000, more preferably 300 to 600 such as biotin, fluorescein,

achieve a consensus behavior. In other words, the oligonucleotide sequences should be sufficiently numerous that several possible probes overlap or fall within a given region that is expected to yield acceptable hybridization efficiency. Since the location of these regions is not known before hand, the best strategy is to equally space the probe sequences along the sequence that is hybridizable to the target sequence. Since regions of acceptable hybridization efficiency are generally on the order of 20 nucleotides in length, a practical strategy is to space the starting nucleotides of the oligonucleotide sequences no more than five basepairs apart. If computation time needed to calculate the predictive parameters is not an issue, then the best strategy is to space the starting nucleotides one nucleotide apart. An important feature of the present invention is to determine oligonucleotides that are clustered along a region of the nucleotide sequence. The individual predictions made for individual oligonucleotide sequences are not very good. However, we have found that the predictions that are experimentally observed tend to form contiguous clusters, while the spurious predictions tend to be solitary. Thus, the number of oligonucleotides should be sufficient to achieve the desired clustering.

Preferably, a set of overlapping sequences is chosen. To this end, the subsequences are chosen so that there is overlap of at least one nucleotide from one oligonucleotide to the next. More preferably, the overlap is two or more nucleotides. Most preferably, the oligonucleotides are spaced one nucleotide apart and the predetermined number is L-N+1 oligonucleotides where L is the length of the nucleotide sequence and N is the length of the oligonucleotides. In the latter situation, the unique oligonucleotides are of identical length N. Thus, a set of overlapping oligonucleotides is a set of oligonucleotides that are subsequences derived from some master sequence by subdividing that sequence in such a way that each subsequence contains either the start or end of at least one other subsequence in the set.

An example of the above for purposes of illustration and not limitation is presented by the sequence ATGGACTTAGCATTCG (SEQ ID NO:3), from which the following set of overlapping oligonucleotides can be identified:

ATGGACTTAGCA (SEQ ID NO:4)
TGGACTTAGCAT (SEQ ID NO:5)
GGACTTAGCATT (SEQ ID NO:6)
GACTTAGCATTC (SEQ ID NO:7)
ACTTAGCATTCG (SEQ ID NO:8)

In this example the overlapping oligonucleotides are spaced one nucleotide apart. In other words, there is overlap of all but one nucleotide from one oligonucleotide to the next. In the example above, the original nucleotide sequence is 16 nucleotides long (L=16). The length of each of the overlapping oligonucleotides is 12 nucleotides long (N=12) and there are L-N+1 = 5 oligonucleotides.

The length of the oligonucleotides may be the same or different and may vary depending on the length of the nucleotide sequence. The length of the oligonucleotides is determined by a practical compromise between the limits of current chemistries for oligonucleotide synthesis and the need for longer oligonucleotides, which exhibit greater binding affinity for the target sequence and are more likely to occur only once in complicated mixtures of polynucleotide targets. Usually, the length of the oligonucleotides is from about 10 to 50 nucleotides, more usually, from about 25 to 35 nucleotides.

In the next step of the method at least one parameter that is independently predictive of the ability of each of the oligonucleotides of the set to hybridize to the target nucleotide sequence is determined and evaluated for each of the above oligonucleotides. Examples of such a parameter, by way of illustration and not limitation, is a parameter selected from the group consisting of composition factors, thermodynamic factors, chemosynthetic efficiencies, kinetic factors and mathematical combinations of these quantities.

The determination of a parameter may be carried out by known methods. For example, melting temperature of the oligonucleotide/target duplex may be determined using the nearest neighbor method and parameters appropriate for the nucleotide acids involved. For DNA/DNA parameters, see J. SantaLucia Jr., *et al.*, (1996) Biochemistry, 35:3555. For RNA/DNA parameters, see N. Sugimoto, *et al.*, (1995) Biochemistry, 34:11211. Briefly, these methods are

based on the observation that the thermodynamics of a nucleic acid duplex can be modeled as the sum of a term arising from the entire duplex and a set of terms arising from overlapping pairs of nucleotides ("nearest neighbor" model). For a discussion of the nearest neighbor see J. SantaLucia Jr., *et al.*, (1996) Biochemistry, *supra*, and N. Sugimoto, *et al.*, (1995) Biochemistry, *supra*. For example, the enthalpy ΔH of the duplex formed by the sequence

ATGGACTTAGCA (SEQ ID NO:4)

and its perfect complement can be approximated by the equation

$$\Delta H \cong H_{init} + H_{AT} + H_{TG} + H_{GG} + H_{GA} + H_{AC} + H_{CT} + H_{TT} + H_{TA} + H_{AG} + H_{GC} + H_{CA} .$$

In the above equation, the term H_{init} is the initiation enthalpy for the entire duplex, while the terms H_{AT}, ..., H_{CA} are the so-called "nearest neighbor" enthalpies. Similar equations can be written for the entropy, for the corresponding quantities for RNA homoduplexes, or for DNA/RNA heteroduplexes. The free energy can then be calculated from the enthalpy, entropy and absolute temperature, as described previously.

Predicted free energy of the most stable intramolecular structure of an oligonucleotide (ΔG_{MFOLD}) may be determined using the nucleic acid folding algorithm MFOLD and parameters appropriate for the oligonucleotide, e.g., DNA or RNA. For MFOLD, see J.A. Jaeger, *et al.*, (1989), *supra*. For DNA folding parameters, see J. SantaLucia Jr., *et al.*, (1996), *supra*. Briefly, these methods operate in two steps. First, a map of all possible compatible intramolecular base pairs is made. Second, the global minimum of the free energy of the various possible base pairing configurations is found, using the nearest neighbor model to estimate the enthalpy and entropy, the user input temperature to complete the calculation of free energy, and a dynamic programming algorithm to find the global minimum. The algorithm is computationally intensive; calculation times scale as the third power of the sequence length.

The following Table 1 summarizes groups of parameters that are independently predictive of the ability of each of the oligonucleotides to hybridize to the target nucleotide sequence together with a reference to methods for their determination. Parameters within a given group are known or expected to be strongly correlated to one another, while parameters in different groups are known or expected to be poorly correlated with one another.

Table 1

Group	Parameter	Source or Reference
I	duplex enthalpy, ΔH	Santa Lucia *et al.*, 1996; Sugimoto *et al.*, 1995
	duplex entropy, ΔS	Santa Lucia *et al.*, 1996; Sugimoto *et al.*, 1995
	duplex free energy, ΔG	$\Delta G=\Delta H-T\Delta S$ (see text)
	melting temperature, T_m	(see text)
	mole fraction (or percent) G+C	self-explanatory
	subsequence duplex enthalpy	Santa Lucia *et al.*, 1996; Sugimoto *et al.*, 1995
	subsequence duplex entropy	Santa Lucia *et al.*, 1996; Sugimoto *et al.*, 1995
	subsequence duplex free energy	$\Delta G=\Delta H-T\Delta S$ (see text)
	subsequence duplex T_m	(see text)
	subsequence duplex mole fraction (or percent) G+C	self-explanatory
II	intramolecular enthalpy, ΔH_{MFOLD}	Jaeger *et al.*, 1989; Santa Lucia *et al.*, 1996
	intramolecular entropy, ΔS_{MFOLD}	Jaeger *et al.*, 1989; Santa Lucia *et al.*, 1996
	intramolecular free energy, ΔG_{MFOLD}	$\Delta G=\Delta H-T\Delta S$ (see text)
	hairpin enthalpy, $\Delta H_{hairpin}$	Jaeger *et al.*, 1989; Santa Lucia *et al.*, 1996
	hairpin entropy, $\Delta S_{hairpin}$	Jaeger *et al.*, 1989; Santa Lucia *et al.*, 1996
	hairpin free energy, $\Delta G_{hairpin}$	$\Delta G=\Delta H-T\Delta S$ (see text)
	intramolecular partition function, Z	$Z = \sum_{k\ structures} \exp\left(-\Delta G^{(k)}_{\text{intramolecular}} / RT\right)$
III	sequence complexity	Altschul *et al.*, 1994
	sequence information content	Altschul *et al.*, 1994
IV	steric factors	molecular modeling or experiment
	molecular dynamic simulation	Weber & Hefland, 1979
	enthalpy, entropy & free energy of activation	measured experimentally
	association & dissociation rates	Patzel & Sczakiel, 1998
V	oligonucleotide chemosynthetic efficiencies	measured experimentally
VI	target synthetic efficiencies	measured experimentally

In a next step of the present method, a subset of oligonucleotides within the predetermined number of unique oligonucleotides is identified based on the above evaluation of the parameter. A number of mathematical approaches may be followed to sort the oligonucleotides based on a parameter. In one approach a cut-off value is established. The cut-off value is adjustable and can be optimized

relative to one or more training data sets. This is done by first establishing some metric for how well a cutoff value is performing; for example, one might use the normalized signal observed for each oligonucleotide in the training set. Once such a metric is established, the cutoff value can be numerically optimized to maximize the value of that metric, using optimization algorithms well known to the art. Alternatively, the cutoff value can be estimated using graphical methods, by graphing the value of the metric as a function of one or more parameters, and then establishing cutoff values that bracket the region of the graph where the chosen metric exceeds some chosen threshold value. In essence, the cut off values are chosen so that the rule set used yields training data that maximizes the inclusion of oligonucleotides that exhibit good hybridization efficiency and minimizes the inclusion of oligonucleotides that exhibit poor hybridization efficiency.

A preferred approach to performing such a graph-based optimization of filter parameters is shown in Fig. 3. In Fig. 3, hybridization data from several different genes have been used to prepare a contour plot of relative hybridization intensity as a function of DNA/RNA heteroduplex melting temperature and free energy of the most stable intramolecular structure of the probe. Contours are shown only for regions for which there are data; the white space outside of the outermost contour indicates that there are no experimental data for that region. The details of how the data were obtained can be found in Example 1 below. A summary of the sequences and number of data points employed is shown in Table 2 below. The measured hybridization intensities for each data set were normalized prior to construction of the contour plot depicted in Fig. 3 by dividing each observed intensity by the maximum intensity observed for that gene. In addition, differences in hybridization salt concentrations and hybridization temperatures were accounted for by using the salt concentration-corrected values of the melting temperatures and by subtracting the hybridization temperature from each predicted melting temperature, respectively. The filter set determined by examination of Fig. 3 is indicated by both the dotted open box in the figure and by the inequalities above the box.

One way in which such a contour plot may be prepared involves the use of an appropriate software application such as Microsoft® Excel® or the like. For

example, the cross-tabulation tool may be used in the Microsoft® Excel® program. Data is accumulated into rectangular bins that are 0.5 kcal ΔG_{MFOLD} wide and 2.5°C T_m wide. In each bin the average values of ΔG_{MFOLD}, $T_m - T_{hyb}$, and the normalized hybridization intensity are calculated. The data is output to the software application DeltaGraph® (Deltapoint, Inc., Monterey, CA) and the contour plot is prepared using the tools and instructions provided.

Table 2

Target (GenBank Accession No.)	Target Strand	No. Data Points	T_{hyb}	[Na^+] Correction
HIV protease-reverse transcriptase (PRT)[a] (M15654)	Sense	1,022	35°C	-1.4°C
HIV protease-reverse transcriptase (PRT)[a] (M15654)	antisense	1,041	30°C	-1.4°C
HIV protease-reverse transcriptase (PRT)[b] (M15654)	Sense	88	35°C	-1.4°C
Human G3PDH (glyceraldehyde-3-dehydrogenase)[b] (X01677)	antisense	93	35°C	-1.4°C
Human p53[b] (X02469)	antisense	93	35°C	-1.4°C
Rabbit β-globin[c] (K03256)	antisense	106	30°C	0°C

[a] Data from Affymetrix GeneChip™ Array
[b] Data from biotinylated probes bound to streptavidin-coated microtiter wells
[c] Literature data: see N. Milner, K. U. Mir & E. M. Southern (1997) *Nature Biotech.* **15**, 537-541.

Once the cut-off value is selected, a subset of oligonucleotides having parameter values greater than or equal to the cut-off value is identified. This refers to the inclusion of oligonucleotides in a subset based on whether the value of a predictive parameter satisfies an inequality.

Examples of identifying a subset of oligonucleotides by establishing cut-off values for predictive parameters are as follows: for melting temperature an inequality might be 60°C ≤ T_m; for predicted free energy an inequality, preferably, might be

$$\Delta G_{MFOLD} \geq -0.4 \frac{kcal}{mole}.$$

In a variation of the above, both a maximum and a minimum cut-off value may be selected. A subset of oligonucleotides is identified whose values fall

within the maximum and minimum values, i.e., values greater than or equal to the minimum cut-off value and less than or equal to the maximum cut-off value. An example of this approach for melting temperature might be the inequality $60°C \leq T_m \leq 85°C$.

With regard to cut off values for T_m the lower limit is most important, and is preferably $T_m = T_{hyb}$, more preferably, $T_m = T_{hyb} + 15°C$. The upper cutoff is important when the sequence region under consideration is unusually rich in G and C, and is preferably $T_m = T_{hyb} + 40°C$. With regard to ΔG_{MFOLD} the cutoff value is usually greater than or equal to –1.0 kcal/mole. As mentioned above, the cutoff values preferably are determined from real data through experimental observations.

In another approach the parameter values may be converted into dimensionless numbers. The parameter value is converted into a dimensionless number by determining a dimensionless score for each parameter resulting in a distribution of scores having a mean value of zero and a standard deviation of one. The dimensionless score is a number that is used to rank some object (such as an oligonucleotide) to which that score relates. A score that has no units (i.e., a pure number) is called a dimensionless score.

In one approach the following equations are used for converting the values of said parameters into dimensionless numbers:

$$s_{i,x} = \frac{x_i - \langle x \rangle}{\sigma_{\{x\}}},$$

where $s_{i,x}$ is the dimensionless score derived from parameter x calculated for oligonucleotide i, x_i is the value of parameter x calculated for oligonucleotide i, $\langle x \rangle$ is the average of parameter x calculated for all of the oligonucleotides under consideration for a given nucleotide sequence target, and $\sigma_{\{x\}}$ is the standard deviation of parameter x calculated for all of the oligonucleotides under consideration for a given nucleotide sequence target, and is given by the equation

$$\sigma_{\{x\}} = \sqrt{\frac{\sum_{j=1}^{M}\left(x_j - \langle x \rangle\right)^2}{M-1}},$$

where M is the number of oligonucleotides. The resulting distribution of scores, *{s}* has a mean value of zero and a standard deviation of one. These properties can be important for a combination of the scores discussed below.

The use of a dimensionless number approach may further include calculating a combination score S_i by evaluating a weighted average of the individual values of the dimensionless scores $s_{i,x}$ by the equation:

$$S_i = \sum_{\{x\}} q_x \, s_{i,x},$$

where q_x is the weight assigned to the score derived from parameter *x*, the individual values of q_x are always greater than zero, and the sum of the weights q_x is unity.

In another variation of the above approach, the method of calculation of the composite parameter is optimized based on the correlation of the individual composite scores to real data, as explained more fully below.

In one approach the calculation of the composite score further involves determining a moving window-averaged combination score $\langle S_i \rangle$ for the *i*th probe by the equation:

$$\langle S_i \rangle = \frac{1}{w} \sum_{j=i-\frac{w-1}{2}}^{i+\frac{w-1}{2}} S_j \,, \; w = \text{an odd integer},$$

where *w* is the length of the window for averaging (i.e., *w* nucleotides long), and then applying a cutoff filter to the value of $\langle S_i \rangle$. This procedure results in smoothing (smoothing procedure) by turning each score into a consensus metric for a set of *w* adjacent oligonucleotide probes. The score, referred to as the "smoothed score," is essentially continuous rather than a few discrete values. The

value of the smoothed score is strongly influenced by clustering of scores with high or low values; window averaging therefore provides a measurement of cluster size.

An advantage of the dimensionless score approach to the probe prediction algorithm is that it is easy to objectively optimize. In one approach to training the algorithm, optimization of the weights q_x above may be performed by varying the values of the weights so that the correlation coefficient $\rho_{\{<Si>\},\{Vi\}}$ between the set of window-averaged combination scores $\{<S_i>\}$ and a set of calibration experimental measurements $\{V_i\}$ is maximized. The correlation coefficient $\rho_{\{<Si>\},\{Vi\}}$ is calculated from the equation

$$\rho_{\{<S_i>\},\{V_i\}} = \left(\frac{1}{M}\right)\frac{\text{Covariance}(\langle S\rangle, V)}{\sigma_{\{<S_i>\}}\,\sigma_{\{V_i\}}},$$

where M is the number of window averaged, combination dimensionless scores and the number of corresponding measurements, the covariance is as defined earlier (see earlier equations) and $\sigma_{\{<Si>\}}$ and $\sigma_{\{Vi\}}$ are the standard deviations of $\{<S_i>\}$ and $\{V_i\}$, as defined previously. An example of this approach is shown in Example 2, below.

In another approach the parameter is derived from one or more factors by mathematical transformation of the factors. This involves the calculation of a new predictive parameter from one or more existing predictive parameters, by means of an equation. For instance, the equilibrium constant K_{open} for formation of an oligonucleotide with no intramolecular structure from its structured form can be calculated from the intramolecular structure free energy ΔG_{MFOLD}, using the equation:

$$K_{open} = \exp\left(\frac{\Delta G_{MFOLD}}{RT}\right).$$

In a next step of the method oligonucleotides in the subset are then identified that are clustered along a region of the nucleotide sequence that is

hybridizable to the target nucleotide sequence. For example, consider a set of overlapping oligonucleotides identified by dividing a nucleotide sequence into subsequences. A subset of the oligonucleotides is obtained as described above. In general, this subset is obtained by applying a rule that rejects some members of the set. For the remaining members of the set, namely, the subset, there will be some average number of nucleotides in the nucleotide sequence between the first nucleotides of adjacent remaining subsequences. If, for some sub-region of the nucleotide sequence, the average number of nucleotides in the nucleotide sequence between the first nucleotides of adjacent remaining subsequences is less than the average for the entire nucleotide sequence, then the oligonucleotides are clustered. The smaller the average number of nucleotides between the first nucleotides of adjacent oligonucleotides, the stronger the clustering. The strongest clustering occurs when there are no intervening nucleotides between adjacent starting nucleotides. In this case, the oligonucleotides are said to be contiguous and may be referred to as contiguous sequence elements or "contigs."

Accordingly, in this step oligonucleotides are sorted based on length of contiguous sequence elements. Oligonucleotides in the subset determined above are identified that are contiguous along a region of the input nucleic acid sequence. The length of each contig that is equal to the number of oligonucleotides in each contig, namely, oligonucleotides from the above step whose complement begin at positions m+1, m+2, ..., m+k in the target sequence, form a contig of length k. Contigs can be identified and contig length can be calculated using, for example, a Visual Basic ® module that can be incorporated into a Microsoft ® Excel workbook.

Cluster size can be defined in several ways:

For contiguous clusters, the size is simply the number of adjacent oligonucleotides in the cluster. Again, this may also be referred to as contiguous sequence elements. The number may also be referred to as "contig length". For example, consider the nucleotide sequence discussed above, namely, ATGGACTTAGCATTCG (SEQ ID NO:3) and the identified set of overlapping oligonucleotides

rhodamine and other dyes, tetracycline and other protein binding molecules, and haptens, *etc.* The small organic molecule can provide a means for attachment of a nucleotide sequence to a label or to a support.

Support or surface -- a porous or non-porous water insoluble material. The surface can have any one of a number of shapes, such as strip, plate, disk, rod, particle, including bead, and the like. The support can be hydrophilic or capable of being rendered hydrophilic and includes inorganic powders such as glass, silica, magnesium sulfate, and alumina; natural polymeric materials, particularly cellulosic materials and materials derived from cellulose, such as fiber containing papers, e.g., filter paper, chromatographic paper, etc.; synthetic or modified naturally occurring polymers, such as nitrocellulose, cellulose acetate, poly (vinyl chloride), polyacrylamide, cross linked dextran, agarose, polyacrylate, polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polymethacrylate, poly(ethylene terephthalate), nylon, poly(vinyl butyrate), etc.; either used by themselves or in conjunction with other materials; glass available as Bioglass, ceramics, metals, and the like. Natural or synthetic assemblies such as liposomes, phospholipid vesicles, and cells can also be employed.

Binding of oligonucleotides to a support or surface may be accomplished by well-known techniques, commonly available in the literature. See, for example, A. C. Pease, *et al.*, Proc. Nat. Acad. Sci. USA, 91:5022-5026 (1994).

Label -- a member of a signal producing system. Usually the label is part of a target nucleotide sequence or an oligonucleotide probe, either being conjugated thereto or otherwise bound thereto or associated therewith. The label is capable of being detected directly or indirectly. Labels include (i) reporter molecules that can be detected directly by virtue of generating a signal, (ii) specific binding pair members that may be detected indirectly by subsequent binding to a cognate that contains a reporter molecule, (iii) oligonucleotide primers that can provide a template for amplification or ligation or (iv) a specific polynucleotide sequence or recognition sequence that can act as a ligand such as for a repressor protein, wherein in the latter two instances the oligonucleotide primer or repressor protein will have, or be capable of having, a reporter molecule. In general, any reporter molecule that is detectable can be used.

The reporter molecule can be isotopic or nonisotopic, usually non-isotopic, and can be a catalyst, such as an enzyme, a polynucleotide coding for a catalyst, promoter, dye, fluorescent molecule, chemiluminescent molecule, coenzyme, enzyme substrate, radioactive group, a small organic molecule, amplifiable polynucleotide sequence, a particle such as latex or carbon particle, metal sol, crystallite, liposome, cell, etc., which may or may not be further labeled with a dye, catalyst or other detectable group, and the like. The reporter molecule can be a fluorescent group such as fluorescein, a chemiluminescent group such as luminol, a terbium chelator such as N-(hydroxyethyl) ethylenediaminetriacetic acid that is capable of detection by delayed fluorescence, and the like.

The label is a member of a signal producing system and can generate a detectable signal either alone or together with other members of the signal producing system. As mentioned above, a reporter molecule can be bound directly to a nucleotide sequence or can become bound thereto by being bound to an sbp member complementary to an sbp member that is bound to a nucleotide sequence. Examples of particular labels or reporter molecules and their detection can be found in U.S. Patent No. 5,508,178 issued April 16, 1996, at column 11, line 66, to column 14, line 33, the relevant disclosure of which is incorporated herein by reference. When a reporter molecule is not conjugated to a nucleotide sequence, the reporter molecule may be bound to an sbp member complementary to an sbp member that is bound to or part of a nucleotide sequence.

Signal Producing System -- the signal producing system may have one or more components, at least one component being the label. The signal producing system generates a signal that relates to the presence or amount of a target polynucleotide in a medium. The signal producing system includes all of the reagents required to produce a measurable signal. Other components of the signal producing system may be included in a developer solution and can include substrates, enhancers, activators, chemiluminescent compounds, cofactors, inhibitors, scavengers, metal ions, specific binding substances required for binding of signal generating substances, and the like. Other components of the signal producing system may be coenzymes, substances that react with enzymic products, other enzymes and catalysts, and the like. The signal producing system

provides a signal detectable by external means, by use of electromagnetic radiation, desirably by visual examination. Signal-producing systems that may be employed in the present invention are those described more fully in U.S. Patent No. 5,508,178, the relevant disclosure of which is incorporated herein by reference.

Ancillary Materials -- Various ancillary materials will frequently be employed in the methods and assays utilizing oligonucleotide probes designed in accordance with the present invention. For example, buffers and salts will normally be present in an assay medium, as well as stabilizers for the assay medium and the assay components. Frequently, in addition to these additives, proteins may be included, such as albumins, organic solvents such as formamide, quaternary ammonium salts, polycations such as spermine, surfactants, particularly non-ionic surfactants, binding enhancers, e.g., polyalkylene glycols, or the like.

DETAILED DESCRIPTION OF THE INVENTION

The invention is directed to methods or algorithms for predicting oligonucleotides specific for a nucleic acid target where the oligonucleotides exhibit a high potential for hybridization. The algorithm uses parameters of the oligonucleotide and the oligonucleotide/target nucleotide sequence duplex, which can be readily predicted from the primary sequences of the target polynucleotide and candidate oligonucleotides. In the methods of the present invention, oligonucleotides are filtered based on one or more of these parameters, then further filtered based on the sizes of clusters of oligonucleotides along the input polynucleotide sequence. The methods or algorithms of the present invention may be carried out using either relatively simple user-written subroutines or publicly available stand-alone software applications (e.g., dynamic programming algorithm for calculating self-structure free energies of oligonucleotides). The parameter calculations may be orchestrated and the filtering algorithms may be implemented using any of a number of commercially available computer programs as a framework such as, e.g., Microsoft® Excel spreadsheet, Microsoft® Access relational database and the like. The basic steps involved in the present methods involve parsing a sequence that is complementary to a target nucleotide sequence into a set of overlapping oligonucleotide sequences, evaluating one or more

parameters for each of the oligonucleotide sequences, said parameter or parameters being predictive of probe hybridization to the target nucleotide sequence, filtering the oligonucleotide sequences based on the values for each parameter, filtering the oligonucleotide sequences based on the length of contiguous sequence elements and ranking the contiguous sequence elements based on their length. We have found that oligonucleotides in the longest contiguous sequence elements generally show the highest hybridization efficiencies.

The present methods are based on our recognition that oligonucleotides showing high hybridization efficiencies tend to form clusters. It is believed that this clustering reflects local regions of the target nucleotide sequence that are unstructured and accessible for oligonucleotide binding. Oligonucleotides that are contiguous along a region of the input nucleic acid sequence are identified. These oligonucleotides are sorted based on the length of the contiguous sequence elements. The sorting approach used in the present invention apparently serves as a surrogate for the calculation of local secondary structure of the target nucleotide sequence. This is supported by our observation that treatments intended to eliminate long-range nucleic acid structure (e.g., random fragmentation) do not eliminate the differences in hybridization yields across oligonucleotide probe arrays. This implies that major determinants of efficient hybridization are local regions of the target sequence. The identification of contiguous sequence elements is a simple and efficient method for recognizing clusters of such determinants and, thus, for identifying oligonucleotide probes that exhibit high hybridization efficiency for a target nucleotide sequence.

As mentioned above one embodiment of the present invention is a method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence. A predetermined number of unique oligonucleotides is identified. The length of the oligonucleotides may be the same or different. The oligonucleotides are unique in that no two of the oligonucleotides are identical. The unique oligonucleotides are chosen to sample the entire length of a nucleotide sequence that is hybridizable with the target nucleotide sequence. The actual number of oligonucleotides is generally determined by the length of the nucleotide sequence and the desired result. The number of oligonucleotides should be sufficient to

ATGGACTTAGCA (SEQ ID NO:4)
TGGACTTAGCAT (SEQ ID NO:5)
GGACTTAGCATT (SEQ ID NO:6)
GACTTAGCATTC (SEQ ID NO:7)
ACTTAGCATTCG (SEQ ID NO:8)

Suppose that, after calculation and evaluation of the predictive parameters, four nucleotides remain:

ATGGACTTAGCA (SEQ ID NO:4) TGGACTTAGCAT (SEQ ID NO:5) GGACTTAGCATT (SEQ ID NO:6)	▌	contig
ACTTAGCATTCG (SEQ ID NO:8)	▌	single oligonucleotide

A "contig" encompassing three of the oligonucleotides of the subset is present together with a single oligonucleotide. The contig length is 3 oligonucleotides.

Alternatively, cluster size at some position in the sequence hybridizable or complementary to the target sequence may be defined as the number of oligonucleotides whose center nucleotides fall inside a region of length M centered about the position in question, divided by M. This definition of clustering allows small gaps in clusters. In the example used above for contiguous clusters, if M was 10, then the cluster size would step through the values 0/10,..., 0/10, 1/10, 2/10, 3/10, 3/10, 4/10, 4/10, 4/10, 4/10, 4/10, 3/10, 2/10, 1/10, 1/10, 0/10 as the center of the window of length 10 passed through the cluster. In each fraction, the numerator is the number of oligonucleotide sequences that have satisfied the filter set and whose central nucleotides are within a window 10 nucleotides long, centered about the nucleotide under consideration. The denominator (10) is simply the window length.

Another alternative is to define the size of a cluster at some position in the sequence hybridizable or complementary to the target sequence as the number of oligonucleotide sequences overlapping that position. This definition is equivalent to the last definition with M set equal to the oligonucleotide probe length and omission of the division by M.

Finally, cluster size can be approximated at each position in a nucleotide sequence by dividing the sequence into oligonucleotides, evaluating a numerical score for each oligonucleotide, and then averaging the scores in the neighborhood

of each position by means of a moving window average as described above. Window averaging has the effect of reinforcing clusters of high or low values around a particular position, while canceling varying values about that position. The window average, therefore, provides a score that is sensitive to both the hybridization potential of a given oligonucleotide and the hybridization potentials of its neighbors.

In a next step of the present method, the oligonucleotides in the subset are ranked. Generally, this ranking is based on the lengths of the clusters or contigs, sizes of the clusters or values of a window averaged score. Oligonucleotides found in the longest contigs or largest clusters, or possessing the highest window averaged scores usually show the highest hybridization efficiencies. Often, the highest signal intensity within the cluster corresponds to the median oligonucleotide of the cluster. However, the peak signal intensity within the contig can be determined experimentally, by sampling the cluster at its first quartile, midpoint and third quartile, measuring the hybridization efficiencies of the sampled oligonucleotides, interpolating or extrapolating the results, predicting the position of the optimal probe, and then iterating the probe design process.

Fig. 1 shows a diagram of an example of the above-described method by way of illustration and not limitation. Referring to Fig. 1 a target sequence of length L from, e.g., a database, is used to generate a sequence that is hybridizable to the target sequence from which candidate oligonucleotide probe sequences are generated. One or more parameters are calculated for each of the oligonucleotide probe sequences. The candidate oligonucleotide probe sequences are filtered based on the values of the parameters. Clustering of the filtered candidate probe sequences is evaluated and the clusters are ranked by size. Then, the oligonucleotide probes are statistically sampled and synthesized. Further evaluation may be made by evaluating the hybridization of the selected oligonucleotide probes in real hybridization experiments. The above process may be reiterated to further define the selection. In this way only a small fraction of the potential oligonucleotide probe candidates are synthesized and tested. This is in sharp contrast to the known method of synthesizing and testing all or a major portion of potential oligonucleotide probes for a given target sequence.

The methods of the present invention are preferably carried out at least in part with the aid of a computer. For example, an IBM® compatible personal computer (PC) may be utilized. The computer is driven by software specific to the methods described herein.

The preferred computer hardware capable of assisting in the operation of the methods in accordance with the present invention involves a system with at least the following specifications: Pentium® processor or better with a clock speed of at least 100 MHz, at least 32 megabytes of random access memory (RAM) and at least 80 megabytes of virtual memory, running under either the Windows 95 or Windows NT 4.0 operating system (or successor thereof).

As mentioned above, software that may be used to carry out the methods may be either Microsoft Excel or Microsoft Access, suitably extended via user-written functions and templates, and linked when necessary to stand-alone programs that calculate specific parameters (e.g., MFOLD for intramolecular thermodynamic parameters). Examples of software programs used in assisting in conducting the present methods may be written, preferably, in Visual BASIC, FORTRAN and C++, as exemplified below in the Examples. It should be understood that the above computer information and the software used herein are by way of example and not limitation. The present methods may be adapted to other computers and software. Other languages that may be used include, for example, PASCAL, PERL or assembly language.

Fig. 2 depicts a more specific approach to a method in accordance with the present invention. Referring to Fig. 2, a sequence of length L is obtained from a database such as GenBank, UniGene or a proprietary sequence database. Probe length N is determined by the user based on the requirements for sensitivity and specificity and the limitations of the oligonucleotide synthetic scheme employed. The probe length and sequence length are used to generate L-N+1 candidate oligonucleotide probes, i.e., from every possible starting position. An initial selection is made based on local sequence predicted thermodynamic properties. To this end, melting temperature T_m and the self-structure free energy ΔG_{MFOLD}, are calculated for each of the potential oligonucleotide probe: target nucleotide sequence complexes. Next, M probes that satisfy T_m and ΔG_{MFOLD} filters are selected. A further selection can be made based on clustering of "good"

parameters. Good parameters are parameters that satisfy all of the filters in the filter set. Clustering is defined by any of the methods described previously; in Fig. 2, the "contig length" definition of clustering is used.

For each of the M oligonucleotide sequences that satisfied all filters the question is asked whether the oligonucleotide sequence immediately following the sequence under consideration is also one of the sequences that satisfied all of the filters. If the answer to this question is NO, then one stores the current value of the contig length counter, resets the counter to zero and proceeds to the next oligonucleotide sequence that satisfied all filters. If the answer to the question is YES, then 1 is added to the contig length counter and, if the counter now equals 1 (i.e., this is the first oligonucleotide probe sequence in the contig), the starting position of the oligonucleotide is stored. One then moves to the next oligonucleotide that satisfied all filters, which, in this case, is the same as the next oligonucleotide before the application of the filter set. The process is repeated until all M filtered oligonucleotide sequences have been examined. In this way, a single pass through the set of M filtered oligonucleotide sequences generates the lengths and starting positions of all contigs.

Next, contigs are ranked based on the lengths of their contiguous sequence elements. Longer contig lengths generally correlate with higher hybridization efficiencies. All oligonucleotides of the higher-ranking contigs may be considered, or candidate oligonucleotide probes may be picked. For example, candidate oligonucleotide probes can be picked one quarter, one half and three quarters of the way through each contig. The latter approach provides local curvature determination after experimental determination of hybridization efficiencies, which allows either interpolation or extrapolation of the positions of the next probes to be synthesized in order to close in on the optimal probe in the region. If the contig brackets the actual peak of hybridization efficiency, the process will converge in 2-3 iterations. If the contig lies to one side of the actual peak, the process will converge in 3-4 iterations.

The above illustrative approach is further described with reference to the following DNA nucleotide sequence, which is the complement of the target RNA nucleotide sequence:

GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA (SEQ ID NO:9).

In the first step of the method, the nucleotide sequence is divided into overlapping oligonucleotides that are 25 nucleotides in length. This length is chosen because it is an effective compromise between the need for sensitivity (enhanced by longer oligonucleotides) and the chemosynthetic efficiency of schemes for synthesis of surface-bound arrays of oligonucleotide probes.

Next, the estimated duplex melting temperatures (T_m) and self-structure free energies (ΔG_{MFOLD}) are calculated for each oligonucleotide in the set of overlapping oligonucleotides. The values are obtained from a user-written function that calculates DNA/RNA heteroduplex thermodynamic parameters (see N. Sugimoto, *et al.*, Biochemistry, 34:11211 (1995)) and a modified version of the program MFOLD that estimates the free energy of the most stable intramolecular

structure of a single stranded DNA molecule (see J.A. Jaeger, *et al.*, (1989), *supra,* respectively. The steps are illustrated below.

```
GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA      (target complement sequence)
```

	T_m (°C)	ΔG_{MFOLD}	
GTCCAAAAAGGGTCAGTCTACCTCC	71.77	-1.20	SEQ ID NO:10
TCCAAAAAGGGTCAGTCTACCTCCC	71.99	-1.20	SEQ ID NO:11
CCAAAAAGGGTCAGTCTACCTCCCG	70.78	-1.20	SEQ ID NO:12
CAAAAAGGGTCAGTCTACCTCCCGC	71.23	-1.20	SEQ ID NO:13
AAAAAGGGTCAGTCTACCTCCCGCC	73.07	-1.20	SEQ ID NO:14
AAAAGGGTCAGTCTACCTCCCGCCA	75.68	-1.20	SEQ ID NO:15
AAAGGGTCAGTCTACCTCCCGCCAT	77.53	-1.20	SEQ ID NO:16
AAGGGTCAGTCTACCTCCCGCCATA	79.03	-1.20	SEQ ID NO:17
AGGGTCAGTCTACCTCCCGCCATAA	79.03	-1.20	SEQ ID NO:18
GGGTCAGTCTACCTCCCGCCATAAA	76.85	-1.20	SEQ ID NO:19
GGTCAGTCTACCTCCCGCCATAAAA	73.10	-0.80	SEQ ID NO:20
GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90	SEQ ID NO:21
TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90	SEQ ID NO:22
CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90	SEQ ID NO:23
AGTCTACCTCCCGCCATAAAAAACT	65.	1.10	SEQ ID NO:24
GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40	SEQ ID NO:25
TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90	SEQ ID NO:26
CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70	SEQ ID NO:27
TACCTCCCGCCATAAAAAACTCATG	62.58	1.10	SEQ ID NO:28
ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40	SEQ ID NO:29
CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10	SEQ ID NO:30
CTCCCGCCATAAAAAACTCATGTTC	63.37	-0.10	SEQ ID NO:31
TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10	SEQ ID NO:32
CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10	SEQ ID NO:33
CCGCCATAAAAAACTCATGTTCAAG	57.98	-0.10	SEQ ID NO:34
CGCCATAAAAAACTCATGTTCAAGA	56.20	-0.10	SEQ ID NO:35

SUB A2

Next, the oligonucleotide sequences are filtered on the basis of T_m. A high and low cut-off value may be selected, for example, $60°C \leq T_m \leq 85°C$. Thus, oligonucleotides having T_m values falling within the above range are retained. Those outside the range are discarded, which is indicated below by lining out of those oligonucleotides and parameter values.

GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA (target complement sequence)

	T_m (°C)	ΔG_{MFOLD}
GTCCAAAAAGGGTCAGTCTACCTCC	71.77	-1.20
TCCAAAAAGGGTCAGTCTACCTCCC	71.99	-1.20
CCAAAAAGGGTCAGTCTACCTCCCG	70.78	-1.20
CAAAAAGGGTCAGTCTACCTCCCGC	71.23	-1.20
AAAAAGGGTCAGTCTACCTCCCGCC	73.07	-1.20
AAAAGGGTCAGTCTACCTCCCGCCA	75.68	-1.20
AAAGGGTCAGTCTACCTCCCGCCAT	77.53	-1.20
AAGGGTCAGTCTACCTCCCGCCATA	79.03	-1.20
AGGGTCAGTCTACCTCCCGCCATAA	79.03	-1.20
GGGTCAGTCTACCTCCCGCCATAAA	76.85	-1.20
GGTCAGTCTACCTCCCGCCATAAAA	73.10	-0.80
GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90
TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90
CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90
AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10
GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40
TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90
CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70
TACCTCCCGCCATAAAAAACTCATG	62.58	1.10
ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40
CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10
CTCCCGCCATAAAAAACTCATGTTC	63.37	-0.10
TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10
CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10
~~CGGCCATAAAAAACTCATGTTCAAG~~	~~57.98~~	-0.10
~~CGGCATAAAAAACTCATGTTCAAGA~~	~~56.20~~	-0.10

SUB A2 cont

Next, the oligonucleotide sequences remaining after the above exercise are filtered on the basis of ΔG_{MFOLD} and are retained if the value is greater than - 0.4. Those oligonucleotides with a ΔG_{MFOLD} less than - 0.4 are discarded, which is indicated below by double lining out of those oligonucleotides and parameter values.

GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA (target complement sequence)

	T_m (°C)	ΔG_{MFOLD}
~~GTCGAAAAAGGGTCAGTCTACGTCG~~	71.77	~~-1.20~~
~~TCGAAAAAGGGTCAGTCTACGTCGG~~	71.99	~~-1.20~~
~~CGAAAAAGGGTCAGTCTACGTCGGG~~	70.78	~~-1.20~~
~~GAAAAAGGGTCAGTCTACGTCGGGG~~	71.23	~~-1.20~~
~~AAAAAGGGTCAGTCTACGTCGGGGG~~	73.07	~~-1.20~~
~~AAAAGGGTCAGTCTACGTCGGGGGA~~	75.68	~~-1.20~~
~~AAAGGGTCAGTCTACGTCGGGGGAT~~	77.53	~~-1.20~~
~~AAGGGTCAGTCTACGTCGGGGGATA~~	79.03	~~-1.20~~
~~AGGGTCAGTCTACGTCGGGGGATAA~~	79.03	~~-1.20~~
~~GGGTCAGTCTACGTCGGGGGATAAA~~	76.85	~~-1.20~~
~~GGTCAGTCTACGTCGGGGGATAAAA~~	73.10	~~-0.80~~
GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90
TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90
CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90
AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10
GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40
TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90
CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70
TACCTCCCGCCATAAAAAACTCATG	62.58	1.10
ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40
CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10
CTCCCGCCATAAAAAACTCATGTTC	63.37	-0.10
TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10
CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10
~~CCGCCATAAAAAACTCATGTTCAAG~~	~~57.98~~	-0.10
~~CGCCATAAAAAACTCATGTTCAAGA~~	~~56.20~~	-0.10

SUB A2 CLEAN

Clusters of retained oligonucleotides are identified and ranked based on cluster size. In this example, a contiguous cluster of 13 retained oligonucleotides is identified by the vertical black bar on the left. Any or all of the oligonucleotides in this cluster may be evaluated experimentally.

GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA (target complement sequence)

	T_m (°C)	ΔG_{MFOLD}
~~GTGGAAAAAGGGTGAGTGTAGGTGG~~	71.77	~~-1.20~~
~~TGGAAAAAGGGTGAGTGTAGGTGGG~~	71.99	~~-1.20~~
~~GGAAAAAGGGTGAGTGTAGGTGGGG~~	70.78	~~-1.20~~
~~GAAAAAGGGTGAGTGTAGGTGGGGG~~	71.23	~~-1.20~~
~~AAAAAGGGTGAGTGTAGGTGGGGGG~~	73.07	~~-1.20~~
~~AAAAGGGTGAGTGTAGGTGGGGGGA~~	75.68	~~-1.20~~
~~AAAGGGTGAGTGTAGGTGGGGGGAT~~	77.53	~~-1.20~~
~~AAGGGTGAGTGTAGGTGGGGGGATA~~	79.03	~~-1.20~~
~~AGGGTGAGTGTAGGTGGGGGGATAA~~	79.03	~~-1.20~~
~~GGGTGAGTGTAGGTGGGGGGATAAA~~	76.85	~~-1.20~~
~~GGTGAGTGTAGGTGGGGGGATAAAA~~	73.10	~~-0.80~~
GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90
TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90
CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90
AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10
GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40
TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90
CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70
TACCTCCCGCCATAAAAAACTCATG	62.58	1.10
ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40
CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10
CTCCCGCCATAAAAAACTCATGTTC	63.37	-0.10
TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10
CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10
~~CCGCCATAAAAAACTCATGTTCAAG~~	~~57.98~~	-0.10
~~CGCCATAAAAAACTCATGTTCAAGA~~	~~56.20~~	-0.10

Alternatively, in one approach the oligonucleotides at the first quartile, the median and the third quartile of the cluster may be selected for experimental evaluation, indicated below by bold print.

GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA (target complement sequence)

	T_m (°C)	ΔG_{MFOLD}
~~GTGCAAAAAGCGTGAGTCTAGCTGC~~	71.77	~~-1.20~~
~~TGCAAAAAGCGTGAGTCTAGCTGCG~~	71.99	~~-1.20~~
~~GCAAAAAGCGTGAGTCTAGCTGCGC~~	70.78	~~-1.20~~
~~CAAAAAGCGTGAGTCTAGCTGCGCG~~	71.23	~~-1.20~~
~~AAAAAGCGTGAGTCTAGCTGCGCGC~~	73.07	~~-1.20~~
~~AAAAGCGTGAGTCTAGCTGCGCGCA~~	75.68	~~-1.20~~
~~AAAGCGTGAGTCTAGCTGCGCGCAT~~	77.53	~~-1.20~~
~~AAGCGTGAGTCTAGCTGCGCGCATA~~	79.03	~~-1.20~~
~~AGCGTGAGTCTAGCTGCGCGCATAA~~	79.03	~~-1.20~~
~~GCGTGAGTCTAGCTGCGCGCATAAA~~	76.85	~~-1.20~~
~~CGTGAGTCTAGCTGCGCGCATAAAA~~	73.10	~~-0.80~~
GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90
TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90
CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90
AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10
GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40
TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90
CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70
TACCTCCCGCCATAAAAAACTCATG	62.58	1.10
ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40
CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10
CTCCCGCCATAAAAAACTCATGTTC	63.37	-0.10
TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10
CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10
~~CCGCCATAAAAAACTCATGTTCAAG~~	~~57.98~~	-0.10
~~CGCCATAAAAAACTCATGTTCAAGA~~	~~56.20~~	-0.10

SUB A2 cmb

In one aspect of the present method, at least two parameters are determined wherein the parameters are poorly correlated with respect to one another. The reason for requiring that the different parameters chosen are poorly correlated with one another is that an additional parameter that is strongly correlated to the original parameter brings no additional information to the prediction process. The correlation to the original parameter is a strong indication that both parameters represent the same physical property of the system. Another way of stating this is that correlated parameters are linearly dependent on one another, while poorly correlated parameters are linearly independent of one another. In practice, the absolute value of the correlation coefficient between any two parameters should be less than 0.5, more preferably, less than 0.25, and, most preferably, as close to zero as possible.

In one preferred approach instead of T_m, for each oligonucleotide/target nucleotide sequence duplex, the difference between the predicted duplex melting temperature corrected for salt concentration and the temperature of hybridization of each of the oligonucleotides with the target nucleotide sequence is determined.

In one aspect the present method comprises determining two parameters at least one of the parameters being the association free energy between a subsequence within each of the oligonucleotides and its complementary sequence on the target nucleotide sequence, or some similar, strongly correlated parameter. The object of this approach is to identify a particularly stable subsequence of the oligonucleotide that might be capable of acting as a nucleation site for the beginning of the heteroduplex formation between the oligonucleotide and the target nucleotide sequence. Such nucleation is believed to be the rate-limiting step for process of heteroduplex formation.

The subsequence within the oligonucleotide is from about 3 to 9 nucleotides in length, usually, 5 to 7 nucleotides in length. The subsequence is at least three nucleotides from the terminus of the oligonucleotide. For support-bound oligonucleotides the subsequence is at least three nucleotides from the free end of the oligonucleotide, i.e., the end that is not attached to the support. Generally, this free end is the 5' end of the oligonucleotide. When the oligonucleotide is attached to a support, the subsequence is at least three nucleotides from the end of the oligonucleotide that is bound to the surface of the support to which the oligonucleotide is attached. Generally, the 3' end of the oligonucleotide is bound to the support.

The predictive parameter can be, for example, either melting temperature or duplex free energy of the subsequence with the target nucleotide sequence. The subsequence with the maximum (melting temperature) or minimum (free energy) value of one of the above parameters is chosen as the representative subsequence for that oligonucleotide probe. For example, if the oligonucleotide is 20 nucleotides in length and a subsequence of 5 nucleotides is chosen, i.e., a 5-mer, then parameter values are calculated for all 5-mer subsequences of the oligonucleotide that do not include the 2 nucleotides at the free end of the oligonucleotide. Where 5' is the free end of the oligonucleotide with designated nucleotide number 1, the values are calculated for all 5-mer subsequences with

starting nucleotides from position number 3 to position number 16. Thus, in this example, parameter values for 14 different subsequences are calculated. The subsequence with the maximum value for the parameter is then assigned as the stability subsequence for the oligonucleotide.

The inclusion of the above determination of a stability subsequence results in the following algorithm for determining the potential of an oligonucleotide to hybridize to a target nucleotide sequence. A predetermined number of unique oligonucleotides are identified within a nucleotide sequence that is hybridizable with said target nucleotide sequence. The oligonucleotides are chosen to sample the entire length of the nucleotide sequence. For each of the oligonucleotides, parameters that are independently predictive of the ability of each of said oligonucleotides to hybridize to said target nucleotide sequence are determined and evaluated. Two parameters that may be used are the thermodynamic parameters of T_m and ΔG_{MFOLD}. These parameters give rise to associated parameter filters. In one approach evaluation of the parameters involves establishing cut-off values as described above. Application of these cut-off values results in the identification of a subset of oligonucleotides for further scrutiny under the algorithm. In accordance with this embodiment of the present invention, there is included a stability subsequence limit in addition to the above. Cutoff values are determined either by means of objective optimization algorithms well known to the art or via graphical estimation methods; both approaches have been described previously in this document. In either case, the optimization of cutoff values involves comparison of predictions to known hybridization efficiency data sets. This process results in objective optimization as it looks at prediction versus experimental results and is otherwise referred to herein as "training the algorithm." The experimental data used to train the algorithm is referred to herein as "training data."

In the present approach filters are assigned to the T_m oligonucleotide probe data. The T_m of each oligonucleotide probe needs to be greater than or equal to the assigned filter (T_m probe limit) to be given a filter score of "1"; otherwise, the filter score is "0". In addition, one can also impose a second filter for this parameter; that is, that the T_m of the oligonucleotide probe also has to be less than a defined upper limit. Filters are also assigned to the ΔG_{MFOLD} data. The

ΔG_{MFOLD} of each oligonucleotide probe should be greater than or equal to the assigned filter (ΔG_{MFOLD} limit) to be given a filter score of "1"; otherwise, the filter score is "0". The filter scores are added. Furthermore, one can also impose a second filter for this parameter; that is, that the ΔG_{MFOLD} also has to be less than a defined upper limit. In accordance with the above discussion stability subsequences are identified. This leads to another filter. Accordingly, filters are assigned to the stability sequence data. The stability subsequence of each oligonucleotide probe needs to be greater than or equal to the assigned filter limit to be given a filter score of "1"; otherwise, the filter score is "0". In addition, one can also impose a second filter for this parameter; that is, that the stability subsequence also has to be less than a defined upper limit. In all cases, the filter values are determined by objective optimization (algorithmic or graphical) of the predictions of the present method versus training data, as described previously.

On the basis of the above filter sets a subset of oligonucleotides within said predetermined number of unique oligonucleotides is identified. Oligonucleotides in the subset are identified that are clustered along a region of the nucleotide sequence that is hybridizable to the target nucleotide sequence. The resulting number of oligonucleotide probe regions is examined. The above filters may then be loosened or tightened by changing the filter limits to obtain more or fewer clusters of oligonucleotides to match the goal, which is set by the needs of the investigator. For instance, a particular application might require that the investigator design 5 non-overlapping probes that efficiently hybridize to a given target sequence.

As mentioned above, the contigs may be selected on the basis of contig length. In another approach, the scores defined above may be summed for cluster size determination. To this end the probe score of the particular filter set (e.g., T_m probe limit, ΔG_{MFOLD} limit and stability sequence limit) is calculated for each oligonucleotide probe. The probe score is the sum of the filter scores. Thus, the probe score is 0 if no parameters pass their respective filters. The probe score is 1, 2 or 3 if one, two or three parameters, respectively, pass their filters for that oligonucleotide probe. This summing is continued for each parameter that is in the current filter set of the algorithm used. For a given algorithm a minimum probe score limit is set. In the current example this limit will be at least 1 and could be 2

or 3 depending on the needs of the investigator, the number of probe clusters required and the results of objective optimizations of algorithm performance against training data. The probe score is compared to this probe score limit. If the probe score of oligonucleotide probe *i* is greater than or equal to the probe score limit, then oligonucleotide probe *i* is assigned a score passed value of 1. Next, a window is chosen for the evaluation of clustering (the "cluster window"). This will be the next filter applied. The cluster window ("*w*") smoothes the score passed values by summing the values in a window *w* nucleotides long, centered about position *i*. The resulting sum is called the cluster sum. Usually, the cluster window is an odd integer, usually 7 or 9 nucleotides. The cluster sum values are then filtered, by comparing to a user-set threshold, cluster filter. If cluster sum is greater than or equal to cluster filter, this filter is passed, and the probe is predicted to hybridize efficiently to its target.

This window summing procedure converts the score for the passed value for each oligonucleotide into a consensus metric for a set of *w* adjacent probes. A "consensus metric" is a measurement that distills a number of values into one consensus value. In this case, the consensus value is calculated by simply summing the individual values. The window summing procedure therefore evaluates a property similar to the contig length metric discussed above. However, the summed score has the advantage of allowing for a few probes within a cluster to have not passed their individual probe score limits. We have found that this allows more observed hybridization peaks to be predicted.

It may be desired in some circumstances to combine the results of multiple algorithm versions. We refer to this operation as "tiling". This may be explained more fully as follows. Tiling generally involves joining together the predicted oligonucleotide probe sets identified by multiple algorithm versions. In the context of the present invention, tiling multiple algorithm versions involves forming the union of multiple sets of predictions. These predictions may arise from different embodiments of the present invention. Alternatively, the different sets of predictions may arise from the same embodiment, but different filter sets. The different filter sets may additionally be restricted to different combinations of parameter values. For instance, one filter set might be used when the predicted

duplex melting temperature T_m is greater than or equal to some value, while another might be used when T_m is below that value.

An example of the logical endpoint of tiling multiple filter sets across different regions of the possible combinations of predictive parameters and then forming the union of the resulting predictions is the contour plot shown in Fig. 3, with the associated rule that "the value of the normalized hybridization intensity associated with a particular combination of (T_m-T_{hyb}) and ΔG_{MFOLD} must be greater than or equal to some threshold value." In this case, the contour at the threshold value becomes the filter. This contour and its interior can be thought of as the union of many small rectangular regions ("tiles"), each of which is bracketed by low and high cutoff values for each of the parameters.

The predictions of different algorithm versions can also be combined by forming the intersection of two or more different predictions. The reliability of predictions within such intersection sets is enhanced because such sets are, by definition, insensitive to changes in the details of the predictive algorithm. Intersection is a useful method for reducing the number of predicted probes when a single algorithm version produces too many candidate probes for efficient experimental evaluation.

The most specific oligonucleotide probe set (i.e., the set least likely to include poor probes) will be the intersection set from multiple algorithms. Clusters that have overlapping oligonucleotide probes from multiple algorithms constitute the intersection set of oligonucleotide probes. The oligonucleotide probe that is in the center of an intersection cluster is chosen. This central oligonucleotide probe may have the highest probability of predicting a peak or, in other words, of binding well to the target nucleotide sequence. Oligonucleotide probes on either side of center, which are still within the intersection cluster, may also be selected. The distance of these "side" oligonucleotide probes from the center generally will be shorter or longer depending upon the length of the cluster.

The most sensitive set of oligonucleotide probes (i.e., the set most likely to include at least one good probe) is generally the union set from multiple algorithms. Clusters that are predicted by at least one type of algorithm constitute the union set of oligonucleotide probes. The oligonucleotide probe in the center of a union cluster is chosen. Oligonucleotide probes on either side of center, which

are still within the union cluster, usually are also chosen. The distance of these side probes from the center will be shorter or longer depending upon the length of the cluster. In summary, the combination of using the stability subsequence parameter, tiling multiple filter sets, and making union and intersection cluster sets of oligonucleotide probes exhibits very high sensitivity and specificity in predicting oligonucleotide probes that effectively hybridize to a target nucleotide sequence of interest.

Another aspect of the present invention is a computer based method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence. A predetermined number of unique oligonucleotides within a nucleotide sequence that is hybridizable with the target nucleotide sequence is identified under computer control. The oligonucleotides are chosen to sample the entire length of the nucleotide sequence. A value is determined and evaluated under computer control for each of the oligonucleotides for at least one parameter that is independently predictive of the ability of each of the oligonucleotides to hybridize to the target nucleotide sequence. The parameter values are stored. Based on the examination of the stored parameter values, a subset of oligonucleotides within the predetermined number of unique oligonucleotides is identified under computer control. Then, oligonucleotides in the subset that are clustered along a region of the nucleotide sequence that is hybridizable to the target nucleotide sequence are identified under computer control.

A computer program is utilized to carry out the above method steps. The computer program provides for input of a target-hybridizable or target-complementary nucleotide sequence, efficient algorithms for computation of oligonucleotide sequences and their associated predictive parameters, efficient, versatile mechanisms for filtering sets of oligonucleotide sequences based on parameter values, mechanisms for computation of the size of clusters of oligonucleotide sequences that pass multiple filters, and mechanisms for outputting the final predictions of the method of the present invention in a versatile, machine-readable or human-readable form.

Another aspect of the present invention is a computer system for conducting a method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence. An input means for introducing a target

nucleotide sequence into the computer system is provided. The input means may permit manual input of the target nucleotide sequence. The input means may also be a database or a standard format file such as GenBank. Also included in the system is means for determining a number of unique oligonucleotide sequences that are within a nucleotide sequence that is hybridizable with the target nucleotide sequence. The oligonucleotide sequences is chosen to sample the entire length of the nucleotide sequence. Suitable means is a computer program or software, which also provides memory means for storing the oligonucleotide sequences. The system also includes means for controlling the computer system to carry out a determination and evaluation for each of the oligonucleotide sequences a value for at least one parameter that is independently predictive of the ability of each of the oligonucleotide sequences to hybridize to the target nucleotide sequence. Suitable means is a computer program or software such as, for example, Microsoft® Excel spreadsheet, Microsoft® Access relational database or the like, which also provides memory means for storing the parameter values. The system further comprises means for controlling the computer to carry out an identification of a subset of oligonucleotide sequences within the number of unique oligonucleotide sequences based on the automated examination of the stored parameter values. Suitable means is a computer program or software, which also allocates memory means for storing the subset of oligonucleotides. The system also includes means for controlling the computer to carry out an identification of oligonucleotide sequences in the subset that are clustered along a region of the nucleotide sequence that is hybridizable to the target nucleotide sequence. Suitable means is a computer program or software, which also allocates memory means for storing the oligonucleotide sequences in the subset. The computer system also includes means for outputting data relating to the oligonucleotide sequences in the subset. Such means may be machine readable or human readable and may be software that communicates with a printer, electronic mail, another computer program, and the like. One particularly attractive feature of the present invention is that the outputting means may communicate directly with software that is part of an oligonucleotide synthesizer. In this way the results of the method of the present invention may be used directly to provide instruction for the synthesis of the desired oligonucleotides.

Another advantage of the present invention is that it may be used to predict efficient hybridization oligonucleotides for each of multiple target sequences. Thus, very large arrays may be constructed and tested with minimal synthesis of oligonucleotides.

EXAMPLES

The invention is demonstrated further by the following illustrative examples. Parts and percentages are by weight unless otherwise indicated. Temperatures are in degrees Centigrade (°C) unless otherwise specified. The following preparations and examples illustrate the invention but are not intended to limit its scope. All reagents used herein were from Amresco, Inc., Solon, Ohio (buffers), Pharmacia Biotech, Piscataway, N.J. (nucleoside triphosphates) or Promega, Madison, Wisconsin (RNA polymerases) unless indicated otherwise.

Example 1

Synopsis: Data from labeled RNA target hybridizations to surface-bound DNA probes directed against 4 different gene sequences were compared to the predictions of the preferred version of the prediction algorithm illustrated by the flow chart in Fig. 2. The RNA targets were sequences derived from the human immunodeficiency virus protease-reverse transcriptase region (HIV PRT; sense-strand target polynucleotide), human glyceraldehyde-3-phosphate dehydrogenase gene (G3PDH; antisense-strand target polynucleotide), human tumor suppressor p53 gene (p53; antisense-strand target polynucleotide) and rabbit β-globin gene (β-globin; antisense-strand target polynucleotide). The GenBank accession numbers for the gene sequences, number of data points collected and temperature of hybridization have all been previously listed in Table 2.

Materials and Methods: Three different experimental systems and two different labeling schemes were used to collect data.

The sequence and hybridization data for β-globin were taken from the literature (see Milner *et al.*, (1997), *supra*; in this experiment, ^{32}P-radiolabeled RNA target was used.

The hybridization data for HIV PRT were obtained using an Affymetrix GeneChip™ HIV PRT-sense probe array (i.e. sense strand target polynucleotide) (GeneChip™ HIV PRT 440s, Affymetrix Corporation, Santa Clara, California) as specified by the manufacturer, except that the fluorescein-labeled RNA target was not fragmented prior to hybridization and that hybridization was performed for 24 hours. The concentration of fluorescein-labeled RNA used was 26.3 nM; label density was approximately 18 fluoresceinated uridyl nucleotides per 1 kilobase (kb) RNA transcript. The raw data were collected by scanning the array with a GeneChip™ Scanner 50 (Affymetrix Corporation, Santa Clara, California), as specified by the manufacturer. The raw data were reduced to a feature-averaged (".CEL") file, using the GeneChip™ software supplied with the scanner. Finally, a table of hybridization intensities for perfect-complement 20-mer probes was constructed using the ASCII feature map file supplied with the GeneChip™ software to connect probe sequences to measured hybridization intensities. The resulting data set contained data for every overlapping 20-mer probe to the target sequence.

The data for G3PDH and p53 were measured using 93-feature arrays constructed using commercially available streptavidin-coated microtiter plates (Pierce Chemical Company, Rockford, IL). Every tenth possible 25-mer probe complementary to each target was synthesized and 3'-biotinylated by a contract synthesis vendor (Operon, Inc., Alameda, CA). The 3'-linked biotin was used to anchor individual probes to microtiter wells, via the well known, strong affinity of streptavidin for biotin. Biotinylated DNA probes were resuspended to a concentration of 10 μM in hybridization buffer (5x sodium chloride-sodium phosphate-disodium ethylenediaminetetraacetate (SSPE), 0.05% Triton X-100, filter-sterilized; 1x SSPE is 150 mM sodium chloride, 10 mM sodium phosphate, 1 mM disodium ethylenediaminetetraacetate (EDTA), pH 7.4). Individual probes were diluted 1:10 in hybridization buffer into specified wells (100 μl total volume per well) of a streptavidin-coated microtiter plate; probes were allowed to bind to the covered plates overnight at 35°C. The other 3 wells of the 96-well microtiter

plate were probe-less controls. The coated plates were washed with 3 x 200 µl of wash buffer (6x SSPE, 0.005% Triton X-100, filter-sterilized). Fluorescein-labeled RNA (100 µl of a 10 nM solution in hybridization buffer) was added to each well. The plates were covered and hybridized at 35°C for 20-24 hours. The hybridized plates were washed with 3 x 200 µl of wash buffer. Label was then released in each well by adding 100 µl of 20 µg/ml RNAase I (Sigma Chemical Company, St. Louis, MO) in Tris-EDTA (TE) (10 mM Tris(hydroxymethyl)aminomethane (Tris), 1 mM EDTA, pH 8.0, sterile) and incubating at 35°C for at least 30 minutes. The fluorescence released from the surface of each well was quantitated with a PerSeptive Biosystems Cytofluor II microtiter plate fluorimeter (PerSeptive Biosystems, Inc., Framingham, MA) using the manufacturer's recommended excitation and emission filter sets for fluorescein. Each plate hybridization was performed in quadruplicate, and the data for each probe were averaged to obtain the hybridization intensity.

Labeled RNA targets specific for G3PDH and p53 were produced via T7 RNA polymerase transcription of DNA templates in the presence of fluorescein-UTP (Boehringer Mannheim Corporation, Indianapolis, IN), using the same method as that outlined by Affymetrix for their GeneChip™ HIV PRT sense probe array. The DNA template for G3PDH was purchased from a commercial source (Clontech, Inc., Palo Alto, CA). The DNA template for p53 was obtained by sub-cloning a PCR fragment from an ATCC-derived reference clone (No. 57254) of human p53 into the commercially-available PCR cloning vector pCR2.1-TOPO (Invitrogen, Inc., Carlsbad, CA), then linearizing the plasmid at the end of the polycloning site opposite the vector-derived T7 promoter.

Probe predictions were performed using a software application (referred to as "p5") that was built atop Microsoft's Access relational database application, using added Visual Basic modules, the TrueDB Grid Pro 5.0 (Apex Software Corporation, Pittsburgh, PA) enhancement to Visual Basic, and a version of the FORTRAN application MFOLD, modified to run in a Windows NT 4.0 environment, as an ActiveX control. The Visual Basic source code for the p5 software application is found in the Microfiche appendix to this specification. The DNA target sequence complements that were input into p5 for division into potential oligonucleotide probe sequences are listed below:

Parent Sequence Accession No.: K03256
Locus: BUNGLOB.DNA (portion of rabbit β -globin)
Length: 122

```
   1 TTCTTCCACA TTCACCTTGC CCCACAGGGC AGTGACCGCA GACTTCTCCT CACTGGACAG
  61 ATGCACCATT CTGTCTGTTT TGGGGGATTG CAAGTAAACA CAGTTGTGTC AAAAGCAAGT
 121 GT SEQ ID NO:36
```

Parent Sequence Accession No.: M15654
Locus: HIV_PRTA.S (HIV PRT antisense; parses into probes specific for sense-strand target)
Length: 1040

```
   1 TGTACTGTCC ATTTATCAGG ATGGAGTTCA TAACCCATCC AAAGGAATGG AGGTTCTTTC
  61 TGATGTTTTT TGTCTGGTGT GGTAAGTCCC CACCTCAACA GATGTTGTCT CAGCTCCTCT
 121 ATTTTTGTTC TATGCTGCCC TATTTCTAAG TCAGATCCTA CATACAAATC ATCCATGTAT
 181 TGATAGATAA CTATGTCTGG ATTTTGTTTT TTAAAAGGCT CTAAGATTTT TGTCATGCTA
 241 CTTTGGAATA TTGCTGGTGA TCCTTTCCAT CCCTGTGGAA GCACATTGTA CTGATATCTA
 301 ATCCCTGGTG TCTCATTGTT TATACTAGGT ATGGTAAATG CAGTATACTT CCTGAAGTCT
 361 TCATCTAAGG GAACTGAAAA ATATGCATCA CCCACATCCA GTACTGTTAC TGATTTTTTC
 421 TTTTTTAACC CTGCGGGATG TGGTATTCCT AATTGAACTT CCCAGAAGTC TTGAGTTCTC
 481 TTATTAAGTT CTCTGAAATC TACTAATTTT CTCCATTTAG TACTGTCTTT TTTCTTTATG
 541 GCAAATACTG GAGTATTGTA TGGATTCTCA GGCCCAATTT TTGAAATTTT CCCTTCCTTT
 601 TCCATTTCTG TACAAATTTC TACTAATGCT TTTATTTTTT CTTCTGTCAA TGGCCATTGT
 661 TTAACTTTTG GGCCATCCAT TCCTGGCTTT AATTTTACTG GTACAGTCTC AATAGGGCTA
 721 ATGGGAAAAT TTAAAGTGCA ACCAATCTGA GTCAACAGAT TTCTTCCAAT TATGTTGACA
 781 GGTGTAGGTC CTACTAATAC TGTACCTATA GCTTTATGTC CACAGATTTC TATGAGTATC
 841 TGATCATACT GTCTTACTTT GATAAAACCT CCAATTCCCC CTATCATTTT TGGTTTCCAT
 901 CTTCCTGGCA AACTCATTTC TTCTAATACT GTATCATCTG CTCCTGTATC TAATAGAGCT
 961 TCCTTTAGTT GCCCCCCTAT CTTTATTGTG ACGAGGGGTC GTTGCCAAAG AGTGATCTGA
1021 GGGAAGTTAA AGGATACAGT SEQ ID NO:37
```

Parent Sequence Accession No.: X01677
Locus: G3PDH (Clontech G3PDH template - parses into probes specific for antisense-strand target)
Length: 999

```
   1 GAAGGTCGGA GTCAACGGAT TTGGTCGTAT TGGGCGCCTG GTCACCAGGG CTGCTTTTAA
  61 CTCTGGTAAA GTGGATATTG TTGCCATCAA TGACCCCTTC ATTGACCTCA ACTACATGGT
 121 TTACATGTTC CAATATGATT CCACCCATGG CAAATTCCAT GGCACCGTCA AGGCTGAGAA
 181 CGGGAAGCTT GTCATCAATG GAAATCCCAT CACCATCTTC CAGGAGCGAG ATCCCTCCAA
 241 AATCAAGTGG GGCGATGCTG GCGCTGAGTA CGTCGTGGAG TCCACTGGCG TCTTCACCAC
 301 CATGGAGAAG GCTGGGGCTC ATTTGCAGGG GGGAGCCAAA AGGGTCATCA TCTCTGCCCC
 361 CTCTGCTGAT GCCCCCATGT TCGTCATGGG TGTGAACCAT GAGAAGTATG ACAACAGCCT
 421 CAAGATCATC AGCAATGCCT CCTGCACCAC CAACTGCTTA GCACCCCTGG CCAAGGTCAT
 481 CCATGACAAC TTTGGTATCG TGGAAGGACT CATGACCACA GTCCATGCCA TCACTGCCAC
 541 CCAGAAGACT GTGGATGGCC CCTCCGGGAA ACTGTGGCGT GATGGCCGCG GGGCTCTCCA
 601 GAACATCATC CCTGCCTCTA CTGGCGCTGC CAAGGCTGTG GGCAAGGTCA TCCCTGAGCT
 661 AGACGGGAAG CTCACTGGCA TGGCCTTCCG TGTCCCCACT GCCAACGTGT CAGTGGTGGA
 721 CCTGACCTGC CGTCTAGAAA AACCTGCCAA ATATGATGAC ATCAAGAAGG TGGTGAAGCA
 781 GGCGTCGGAG GGCCCCCTCA AAGGCATCCT GGGCTACACT GAGCACCAGG TGGTCTCCTC
 841 TGACTTCAAC AGCGACACCC ACTCCTCCAC CTTTGACGCT GGGGCTGGCA TTGCCCTCAA
 901 CGACCACTTT GTCAAGCTCA TTTCCTGGTA TGACAACGAA TTTGGCTACA GCAACAGGGT
 961 GGTGGACCTC ATGGCCCACA TGCTATAGTG AGTCGTATT SEQ ID NO:38
```

Parent Sequence Accession No.: X54156
Locus: HSP53PCRa (p53 template - parses into probes specific for antisense-strand target)
Length: 1049

```
   1 GAGGTGCGTG TTTGTGCCTG TCCTGGGAGA GACCGGCGCA CAGAGGAAGA GAATCTCCGC
  61 AAGAAAGGGG AGCCTCACCA CGAGCTGCCC CCAGGGAGCA CTAAGCGAGC ACTGCCCAAC
 121 AACACCAGCT CCTCTCCCCA GCCAAAGAAG AAACCACTGG ATGGAGAATA TTTCACCCTT
 181 CAGATCCGTG GGCGTGAGCG CTTCGAGATG TTCCGAGAGC TGAATGAGGC CTTGGAACTC
 241 AAGGATGCCC AGGCTGGGAA GGAGCCAGGG GGGAGCAGGG CTCACTCCAG CCACCTGAAG
 301 TCCAAAAAGG GTCAGTCTAC CTCCCGCCAT AAAAAACTCA TGTTCAAGAC AGAAGGGCCT
 361 GACTCAGACT GACATTCTCC ACTTCTTGTT CCCCACTGAC AGCCTCCCTC CCCCATCTCT
 421 CCCTCCCCTG CCATTTTGGG TTTTGGGTCT TTGAACCCTT GCTTGCAATA GGTGTGCGTC
 481 AGAAGCACCC AGGACTTCCA TTTGCTTTGT CCCGGGGCTC CACTGAACAA GTTGGCCTGC
 541 ACTGGTGTTT TGTTGTGGGG AGGAGGATGG GGAGTAGGAC ATACCAGCTT AGATTTTAAG
 601 GTTTTTACTG TGAGGGATGT TTGGGAGATG TAAGAAATGT TCTTGCAGTT AAGGGTTAGT
 661 TTACAATCAG CCACATTCTA GGTAGGTAGG GGCCCACTTC ACCGTACTAA CCAGGGAAGC
 721 TGTCCCTCAT GTTGAATTTT CTCTAACTTC AAGGCCCATA TCTGTGAAAT GCTGGCATTT
 781 GCACCTACCT CACAGAGTGC ATTGTGAGGG TTAATGAAAT AATGTACATC TGGCCTTGAA
 841 ACCACCTTTT ATTACATGGG GTCTAAAACT TGACCCCCTT GAGGGTGCCT GTTCCCTCTC
 901 CCTCTCCCTG TTGGCTGGTG GGTTGGTAGT TTCTACAGTT GGGCAGCTGG TTAGGTAGAG
 961 GGAGTTGTCA AGTCTTGCTG GCCCAGCCAA ACCCTGTCTG ACAACCTCTT GGTCGACCTT
1021 AGTACCTAAA AGGAAATCTC ACCCCATCC SEQ ID NO:39
```

The sequences indicated above, which are complements of the target sequences, were divided into overlapping oligonucleotide sequences with one nucleotide between starting positions. The oligonucleotide sequence lengths were 17 (rabbit β-globin), 20 (HIV PRT) or 25 (G3PDH; p53). The oligonucleotide sequence lengths were dictated by the probe lengths used in the experiments to which the predictions were compared. The RNA target concentrations used to calculate predicted RNA/DNA duplex melting temperatures were 100 pM (rabbit β-globin), 26.3 nM (HIV PRT) and 10 nM (G3PDH; p53). These were also dictated by experimental conditions for the comparison data. The cut-off filter used for the predicted free energy of the most stable probe sequence intramolecular structure, ΔG_{MFOLD}, was

$$\Delta G_{MFOLD} \geq -0.4 \frac{\text{kcal}}{\text{mole}}.$$

The filter condition used for the predicted RNA/DNA duplex melting temperature was

$$25°C \leq T_m + 16.6 \log([Na^+]) - T_{hyb} \leq 50°C,$$

where T_m is the target concentration-dependent value of the predicted RNA/DNA duplex melting temperature before correction for salt concentration, the term "16.6 log([Na^+])" corrects the melting temperature for salt effects, and T_{hyb} is the hybridization temperature. The values of the salt correction term and T_{hyb} have already been listed in Table 2. For convenient use within p5, the above condition was algebraically rearranged into the equivalent form

$$25°C - 16.6\log([Na^+]) + T_{hyb} \le T_m \le 50°C - 16.6\log([Na^+]) + T_{hyb}.$$

Clusters were ranked according to the number of contiguous oligonucleotide sequences that passed through the filter set ("contig" length).

Results: The detailed analysis results for rabbit β-globin are presented in Table 3; a graphical summary of the results is shown in Fig. 4. In Table 3, values of T_m and ΔG_{MFOLD} that were excluded by the filter set are shown with a line through them, and table entries for contig length are shown in gray when the oligonucleotide sequence in question was not in a contig. The top 20% of the observed hybridization intensities are shown underlined.

Table 3

Position	Oligonucleotide Sequence	SEQ ID NO:	T_m (°C)	ΔG_{MFOLD} (kcal/mole)	Contig Length	Hybridization Intensity (Milner *et al.*, 1997)
1	TTCTTCCACATTCACCT	40	~~53.62~~	5.00		100
2	TCTTCCACATTCACCTT	41	~~53.62~~	5.00		130
3	CTTCCACATTCACCTTG	42	~~52.19~~	0.90		130
4	TTCCACATTCACCTTGC	43	~~54.50~~	0.50		200
5	TCCACATTCACCTTGCC	44	58.46	0.50	7	120
6	CCACATTCACCTTGCCC	45	61.10	0.50	7	180
7	CACATTCACCTTGCCCC	46	61.10	0.50	7	230
8	ACATTCACCTTGCCCCA	47	61.10	0.50	7	220
9	CATTCACCTTGCCCCAC	48	61.10	0.90	7	320
10	ATTCACCTTGCCCCACA	49	61.10	0.70	7	310
11	TTCACCTTGCCCCACAG	50	61.33	0.50	7	320
12	TCACCTTGCCCCACAGG	51	63.70	~~-0.60~~		390
13	CACCTTGCCCCACAGGG	52	64.85	~~-1.60~~		410
14	ACCTTGCCCCACAGGGC	53	68.01	~~-4.10~~		240
15	CCTTGCCCCACAGGGCA	54	68.63	~~-5.40~~		50
16	CTTGCCCCACAGGGCAG	55	64.95	~~-5.60~~		20
17	TTGCCCCACAGGGCAGT	56	66.31	~~-5.60~~		20
18	TGCCCCACAGGGCAGTG	57	65.79	~~-5.40~~		20
19	GCCCCACAGGGCAGTGA	58	67.37	~~-4.10~~		20
20	CCCCACAGGGCAGTGAC	59	63.42	~~-1.60~~		40
21	CCCACAGGGCAGTGACC	60	63.42	~~-1.40~~		20
22	CCACAGGGCAGTGACCG	61	59.85	~~-1.40~~		20
23	CACAGGGCAGTGACCGC	62	60.14	~~-1.00~~		20
24	ACAGGGCAGTGACCGCA	63	60.14	~~-0.50~~		20
25	CAGGGCAGTGACCGCAG	64	59.76	~~-0.50~~		30
26	AGGGCAGTGACCGCAGA	65	59.83	~~-0.50~~		20
27	GGGCAGTGACCGCAGAC	66	60.22	~~-0.50~~		30
28	GGCAGTGACCGCAGACT	67	59.53	~~-0.50~~		30
29	GCAGTGACCGCAGACTT	68	57.06	~~-0.40~~		30
30	CAGTGACCGCAGACTTC	69	~~53.99~~	~~-0.40~~		40
31	AGTGACCGCAGACTTCT	70	~~54.71~~	-0.20		40
32	GTGACCGCAGACTTCTC	71	55.99	0.60	7	100
33	TGACCGCAGACTTCTCC	72	57.01	0.60	7	120
34	GACCGCAGACTTCTCCT	73	59.22	0.60	7	180
35	ACCGCAGACTTCTCCTC	74	59.28	0.60	7	210
36	CCGCAGACTTCTCCTCA	75	60.07	0.60	7	200
37	CGCAGACTTCTCCTCAC	76	56.34	0.60	7	190
38	GCAGACTTCTCCTCACT	77	57.79	0.60	7	240
39	CAGACTTCTCCTCACTG	78	~~52.93~~	0.60		240
40	AGACTTCTCCTCACTGG	79	~~54.41~~	0.00		340

Table 3

Position	Oligonucleotide Sequence	SEQ ID NO:	T_m (°C)	ΔG_{MFOLD} (kcal/mole)	Contig Length	Hybridization Intensity (Milner *et al.*, 1997)
41	GACTTCTCCTCACTGGA	80	55.77	-1.40		340
42	ACTTCTCCTCACTGGAC	81	54.85	-1.60		240
43	CTTCTCCTCACTGGACA	82	55.75	-1.60		240
44	TTCTCCTCACTGGACAG	83	53.66	-1.60		120
45	TCTCCTCACTGGACAGA	84	54.82	-1.60		100
46	CTCCTCACTGGACAGAT	85	53.36	-1.60		110
47	TCCTCACTGGACAGATG	86	51.10	-1.40		80
48	CCTCACTGGACAGATGC	87	54.25	0.00		240
49	CTCACTGGACAGATGCA	88	51.26	0.20		90
50	TCACTGGACAGATGCAC	89	49.63	0.20		30
51	CACTGGACAGATGCACC	90	52.74	0.50		100
52	ACTGGACAGATGCACCA	91	52.74	-0.50		80
53	CTGGACAGATGCACCAT	92	52.18	-1.00		90
54	TGGACAGATGCACCATT	93	50.39	-0.80		80
55	GGACAGATGCACCATTC	94	51.75	0.30		180
56	GACAGATGCACCATTCT	95	51.05	-0.10		220
57	ACAGATGCACCATTCTG	96	49.56	-1.80		120
58	CAGATGCACCATTCTGT	97	52.19	-2.10		120
59	AGATGCACCATTCTGTC	98	52.06	-0.10		250
60	GATGCACCATTCTGTCT	99	54.18	0.30		520
61	ATGCACCATTCTGTCTG	100	52.60	0.40		980
62	TGCACCATTCTGTCTGT	101	56.05	0.20	2	780
63	GCACCATTCTGTCTGTT	102	56.52	0.20	2	810
64	CACCATTCTGTCTGTTT	103	52.06	0.20		220
65	ACCATTCTGTCTGTTTT	104	50.83	0.20		120
66	CCATTCTGTCTGTTTTG	105	50.18	0.20		120
67	CATTCTGTCTGTTTTGG	106	48.42	0.60		160
68	ATTCTGTCTGTTTTGGG	107	49.91	1.70		310
69	TTCTGTCTGTTTTGGGG	108	53.10	1.70		250
70	TCTGTCTGTTTTGGGGG	109	55.90	1.70	2	80
71	CTGTCTGTTTTGGGGGA	110	55.91	1.40	2	30
72	TGTCTGTTTTGGGGGAT	111	53.55	0.90		50
73	GTCTGTTTTGGGGGATT	112	54.00	0.90		10
74	TCTGTTTTGGGGGATTG	113	50.50	1.10		10
75	CTGTTTTGGGGGATTGC	114	53.77	2.20		10
76	TGTTTTGGGGGATTGCA	115	53.04	1.20		10
77	GTTTTGGGGGATTGCAA	116	51.01	0.00		5
78	TTTTGGGGGATTGCAAG	117	47.99	-0.20		5
79	TTTGGGGGATTGCAAGT	118	50.80	-0.20		5
80	TTGGGGGATTGCAAGTA	119	49.80	0.00		5

Table 3

Position	Oligonucleotide Sequence	SEQ ID NO:	T_m (°C)	ΔG_{MFOLD} (kcal/mole)	Contig Length	Hybridization Intensity (Milner *et al.*, 1997)
81	TGGGGGATTGCAAGTAA	120	~~47.55~~	1.20		5
82	GGGGGATTGCAAGTAAA	121	~~45.76~~	1.40		5
83	GGGGATTGCAAGTAAAC	122	~~43.54~~	1.40		5
84	GGGATTGCAAGTAAACA	123	~~42.32~~	1.30		5
85	GGATTGCAAGTAAACAC	124	~~40.11~~	0.90		5
86	GATTGCAAGTAAACACA	125	~~38.94~~	0.50		5
87	ATTGCAAGTAAACACAG	126	~~37.61~~	0.50		5
88	TTGCAAGTAAACACAGT	127	~~40.35~~	0.50		5
89	TGCAAGTAAACACAGTT	128	~~40.35~~	0.30		5
90	GCAAGTAAACACAGTTG	129	~~40.35~~	0.10		10
91	CAAGTAAACACAGTTGT	130	~~38.98~~	-0.30		5
92	AAGTAAACACAGTTGTG	131	~~37.40~~	~~-0.90~~		5
93	AGTAAACACAGTTGTGT	132	~~42.02~~	~~-2.30~~		5
94	GTAAACACAGTTGTGTC	133	~~43.15~~	~~-2.50~~		5
95	TAAACACAGTTGTGTCA	134	~~41.73~~	~~-2.50~~		5
96	AAACACAGTTGTGTCAA	135	~~40.67~~	~~-2.50~~		5
97	AACACAGTTGTGTCAAA	136	~~40.67~~	~~-2.50~~		5
98	ACACAGTTGTGTCAAAA	137	~~40.67~~	~~-2.30~~		10
99	CACAGTTGTGTCAAAAG	138	~~40.20~~	~~-1.20~~		15
100	ACAGTTGTGTCAAAAGC	139	~~42.93~~	~~-0.50~~		30
101	CAGTTGTGTCAAAAGCA	140	~~43.99~~	0.20		25
102	AGTTGTGTCAAAAGCAA	141	~~40.67~~	-0.10		25
103	GTTGTGTCAAAAGCAAG	142	~~40.67~~	-0.30		20
104	TTGTGTCAAAAGCAAGT	143	~~40.67~~	-0.10		120
105	TGTGTCAAAAGCAAGTG	144	~~40.40~~	0.50		20

In Fig. 4, the hybridization intensity observed experimentally is plotted as a function of oligonucleotide starting position in the target-complementary sequence that was input into p5. The identified contigs are plotted as horizontal bars, with the contig rank (by length) shown in parentheses next to each bar. It is clear from Table 3 and Fig. 4 that the prediction algorithm identified contigs that overlap all of the "top 20%" hybridization intensity peaks observed. Iterative experimental improvement of these predictions would converge on each of the observed intensity maxima in 3-4 iterations.

Prediction worksheets for HIV PRT, G3PDH and p53 were prepared in a manner similar to that for rabbit β-globin as shown in Table 3, except that the probes were longer as indicated above and that approximately 1,000 probes were

analyzed for each of these genes. The results of these analyses are shown in Fig. 5 (HIV PRT), Fig. 6 (G3PDH) and Fig. 7 (p53). In Fig. 5, data are plotted for all possible 20-mer oligonucleotide probes. In Figs. 6 and 7, data were available for only every 10th 25-mer probe, and the actual data points are plotted as open diamonds.

It is clear from Figs. 5-7 that the hybridization efficiency prediction algorithm of the present invention performed well in the task of identifying regions with observed high hybridization intensity. In each case, the 4 longest contigs point to good-to-excellent regions for experimental investigation. It should be noted that the contigs usually bracket observed intensity peaks; experimental iterative refinement would therefore be expected to converge in 2-3 iterations. By this is meant that certain oligonucleotides from the identified contigs are prepared and subjected to evaluation in actual hybridization experiments. Based on the results of such experiments, the observed signal is evaluated to determine whether the oligonucleotides are hybridizing to the left of, the right of, or on the center of a peak with respect to the graphed data. The next iteration is carried out to experimentally evaluate the hybridization efficiency of probes that are inferred to lie closer to the peak of hybridization efficiency, based on the data from the previous iteration. Iteration is continued until the signal level is deemed acceptable by the user, or the local hybridization efficiency maximum is reached (i.e. the best probe in the cluster identified by the method of the current invention has been experimentally identified). A detailed illustration of this process is shown in Example 3.

It should be noted that clusters of predictions that overlap the maxima of observed peaks of hybridization efficiency will often yield user-acceptable probes on the first iteration. Thus, the method of the present invention is much more efficient than current methods in which every potential probe is synthesized. For instance, in the HIV PRT example shown in Fig. 5, at least 3 good probes would be identified after synthesis of ~10 test probes (i.e. statistical sampling of the 3 longest contigs). This is much more efficient than the ~1,000 probes represented by the data in Fig. 5.

Example 2

Synopsis: Data from a labeled RNA target hybridization to an Affymetrix GeneChip™ HIV PRT-sense probe array (GeneChip™ HIV PRT 440s, Affymetrix Corporation, Santa Clara, CA) were compared to the predictions of the window-averaged composite dimensionless score version of the method of the present invention.

Materials and Methods: Data were obtained as described for the Affymetrix GeneChip™ HIV PRT-sense probe array (GeneChip™ HIV PRT 440s, Affymetrix Corporation, Santa Clara, California) in Example 1. The DNA sequence (SEQ ID NO: 37) complementary to the fluorescein-labeled RNA target was divided into overlapping 20-mer oligonucleotide sequences spaced one nucleotide apart, using the prototype application p5; p5 was also used to calculate the predicted values of the RNA/DNA heteroduplex melting temperature (T_m) and the free energy of the most stable predicted probe intramolecular structure, ΔG_{MFOLD}, as described in Example 1. The probe sequences and parameter values were then transferred to a Microsoft Excel spreadsheet, which was used to complete the predictions of efficient and inefficient probes. The weight was obtained by optimizing the performance of the algorithm with the data of Milner *et al.*, *supra*, as the training data using the Microsoft® Excel® spreadsheet software. The composite score was calculated using a weight of 0.62 for the dimensionless T_m score and a weight of 0.38 for the ΔG_{MFOLD} dimensionless score. The windowed-averaging was performed using a window width of 7 and Microsoft® Excel® spreadsheet software. Finally, the oligonucleotide sequences having the top 10% of the window-averaged composite dimensionless scores were predicted to be efficient probes, while the oligonucleotide sequences having the bottom 10% of the window-averaged composite dimensionless scores were predicted to be inefficient probes.

Results: The calculated parameters and scores are shown in Table 4; the algorithm predictions are also shown diagrammatically in Figure 8. In Table 4, window-averaged composite score values that were in the top 10% of the

distribution of values are shown in bold type, values that were in the bottom 10% are shown in italics, and all other values are shown with a line through them. It is clear from both Table 4 and Figure 8 that the window-averaged composite dimensionless score embodiment of the current invention correctly predicted both efficient and inefficient hybridization probes for HIV PRT sense-strand RNA. As in Example 1, statistical sampling of contiguous stretches of predicted "good" probes would lead to convergence of the design process to the best probes in each region in 2-4 design iterations.

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
1	GTACTGTCCATTTATCAGGA	145	64.16	-0.10	0.557	-0.199	0.269		1152.2
2	TACTGTCCATTTATCAGGAT	146	60.91	-0.40	0.080	-0.460	-0.125		1040.7
3	ACTGTCCATTTATCAGGATG	147	61.41	-0.90	0.152	-0.895	-0.246		291.9
4	CTGTCCATTTATCAGGATGG	148	63.46	-0.90	0.453	-0.895	-0.059	~~-0.168~~	221.8
5	TGTCCATTTATCAGGATGGA	149	62.82	-0.90	0.360	-0.895	-0.117	~~-0.281~~	148.3
6	GTCCATTTATCAGGATGGAG	150	63.15	-1.90	0.408	-1.764	-0.418	~~-0.308~~	84.6
7	TCCATTTATCAGGATGGAGT	151	63.15	-2.10	0.408	-1.938	-0.484	~~-0.252~~	128.7
8	CCATTTATCAGGATGGAGTT	152	62.03	-1.90	0.245	-1.764	-0.519	~~-0.242~~	94.6
9	CATTTATCAGGATGGAGTTC	153	59.53	-0.60	-0.122	-0.634	-0.317	~~-0.236~~	157.5
10	ATTTATCAGGATGGAGTTCA	154	59.53	0.80	-0.122	0.583	0.146	~~-0.227~~	316.9
11	TTTATCAGGATGGAGTTCAT	155	59.53	0.40	-0.122	0.236	0.014	~~-0.194~~	360.2
12	TTATCAGGATGGAGTTCATA	156	58.58	0.40	-0.262	0.236	-0.073	~~-0.105~~	403.8
13	TATCAGGATGGAGTTCATAA	157	56.21	0.20	-0.609	0.062	-0.354	~~-0.014~~	382.5
14	ATCAGGATGGAGTTCATAAC	158	57.34	0.20	-0.444	0.062	-0.252	~~-0.004~~	324.4
15	TCAGGATGGAGTTCATAACC	159	61.25	0.20	0.129	0.062	0.104	~~-0.035~~	320.5
16	CAGGATGGAGTTCATAACCC	160	63.57	0.20	0.470	0.062	0.315	~~-0.101~~	238.9
17	AGGATGGAGTTCATAACCCA	161	63.57	-0.10	0.470	-0.199	0.216	~~-0.157~~	202.3
18	GGATGGAGTTCATAACCCAT	162	63.34	-1.30	0.436	-1.243	-0.202	~~-0.120~~	113.6
19	GATGGAGTTCATAACCCATC	163	62.24	-2.00	0.275	-1.851	-0.533	~~-0.099~~	97.7
20	ATGGAGTTCATAACCCATCC	164	64.62	-3.30	0.624	-2.982	-0.746	~~-0.100~~	143.3
21	TGGAGTTCATAACCCATCCC	165	68.18	-2.00	1.146	-1.851	0.007	~~-0.109~~	484.6
22	GGAGTTCATAACCCATCCCA	166	69.39	-1.60	1.324	-1.504	0.249	~~-0.058~~	857.6
23	GAGTTCATAACCCATCCCAA	167	64.93	-0.20	0.670	-0.286	0.307	~~0.053~~	991.4
24	AGTTCATAACCCATCCCAAA	168	61.82	0.20	0.213	0.062	0.155	~~0.173~~	907.0
25	GTTCATAACCCATCCCAAAG	169	61.82	0.20	0.213	0.062	0.155	~~0.137~~	887.9
26	TTCATAACCCATCCCAAAGG	170	61.36	0.60	0.145	0.410	0.246	~~0.053~~	1015.3

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
27	TCATAACCCATCCCAAAGGA	171	62.21	-0.10	0.270	-0.199	0.092	~~-0.040~~	279.7
28	CATAACCCATCCCAAAGGAA	172	59.26	-0.30	-0.163	-0.373	-0.243	~~-0.124~~	210.7
29	ATAACCCATCCCAAAGGAAT	173	58.19	-0.30	-0.320	-0.373	-0.340	~~-0.204~~	179.9
30	TAACCCATCCCAAAGGAATG	174	58.13	-0.30	-0.328	-0.373	-0.345	~~-0.309~~	91.8
31	AACCCATCCCAAAGGAATGG	175	60.78	-1.30	0.061	-1.243	-0.435	~~-0.412~~	44.6
32	ACCCATCCCAAAGGAATGGA	176	63.69	-2.00	0.487	-1.851	-0.401	~~-0.488~~	42.9
33	CCCATCCCAAAGGAATGGAG	177	63.40	-2.20	0.445	-2.025	-0.494	~~-0.542~~	45.0
34	CCATCCCAAAGGAATGGAGG	178	62.34	-2.30	0.290	-2.112	-0.623	~~-0.579~~	45.3
35	CATCCCAAAGGAATGGAGGT	179	61.72	-2.60	0.199	-2.373	-0.778	~~-0.587~~	47.9
36	ATCCCAAAGGAATGGAGGTT	180	60.90	-2.20	0.079	-2.025	-0.721	~~-0.580~~	49.2
37	TCCCAAAGGAATGGAGGTTC	181	62.24	-2.20	0.274	-2.025	-0.600	~~-0.585~~	74.2
38	CCCAAAGGAATGGAGGTTCT	182	62.71	-2.00	0.344	-1.851	-0.490	~~-0.572~~	125.5
39	CCAAAGGAATGGAGGTTCTT	183	59.47	-0.70	-0.132	-0.721	-0.356	~~-0.485~~	183.3
40	CAAAGGAATGGAGGTTCTTT	184	56.10	-0.30	-0.627	-0.373	-0.530	~~-0.380~~	261.4
41	AAAGGAATGGAGGTTCTTTC	185	56.11	-0.30	-0.625	-0.373	-0.529	~~-0.277~~	518.3
42	AAGGAATGGAGGTTCTTTCT	186	60.05	-0.30	-0.046	-0.373	-0.170	~~-0.206~~	716.5
43	AGGAATGGAGGTTCTTTCTG	187	62.09	-0.30	0.253	-0.373	0.015	~~-0.164~~	1056.0
44	GGAATGGAGGTTCTTTCTGA	188	63.23	-0.30	0.420	-0.373	0.119	~~-0.025~~	1084.3
45	GAATGGAGGTTCTTTCTGAT	189	60.56	0.10	0.028	-0.025	0.008	~~0.119~~	1241.1
46	AATGGAGGTTCTTTCTGATG	190	59.12	0.30	-0.183	0.149	-0.057	~~0.217~~	1278.8
47	ATGGAGGTTCTTTCTGATGT	191	64.58	0.30	0.618	0.149	0.440	~~0.258~~	1616.0
48	TGGAGGTTCTTTCTGATGTT	192	64.98	0.30	0.677	0.149	0.476	~~0.270~~	1677.5
49	GGAGGTTCTTTCTGATGTTT	193	65.49	0.30	0.751	0.149	0.522	~~0.300~~	1963.1
50	GAGGTTCTTTCTGATGTTTT	194	63.04	0.30	0.392	0.149	0.300	~~0.301~~	2126.1
51	AGGTTCTTTCTGATGTTTTT	195	61.97	0.30	0.235	0.149	0.202	~~0.231~~	2143.3
52	GGTTCTTTCTGATGTTTTTT	196	62.11	0.30	0.256	0.149	0.215	~~0.180~~	3540.6
53	GTTCTTTCTGATGTTTTTTG	197	59.21	0.30	-0.170	0.149	-0.049	~~0.164~~	1728.7

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
54	TTCTTTCTGATGTTTTTTGT	198	59.21	0.30	-0.170	0.149	-0.049	~~0.151~~	1364.3
55	TCTTTCTGATGTTTTTTGTC	199	60.35	0.50	-0.002	0.323	0.121	~~0.183~~	1788.4
56	CTTTCTGATGTTTTTTGTCT	200	60.96	1.20	0.086	0.931	0.407	~~0.253~~	2670.9
57	TTTCTGATGTTTTTTGTCTG	201	58.76	1.20	-0.235	0.931	0.208	~~0.338~~	3336.2
58	TTCTGATGTTTTTTGTCTGG	202	61.17	1.20	0.118	0.931	0.427	~~0.440~~	6683.6
59	TCTGATGTTTTTTGTCTGGT	203	64.20	1.20	0.562	0.931	0.702	~~0.537~~	10227.0
60	CTGATGTTTTTTGTCTGGTG	204	62.51	1.20	0.315	0.931	0.549	~~0.625~~	10965.0
61	TGATGTTTTTTGTCTGGTGT	205	63.80	1.20	0.504	0.931	0.666	~~0.778~~	11133.0
62	GATGTTTTTTGTCTGGTGTG	206	63.80	1.60	0.504	1.279	0.798	**0.894**	11503.0
63	ATGTTTTTTGTCTGGTGTGG	207	65.18	1.90	0.705	1.540	1.023	**0.894**	9492.8
64	TGTTTTTTGTCTGGTGTGGT	208	68.78	1.70	1.234	1.366	1.284	**0.914**	10704.0
65	GTTTTTTGTCTGGTGTGGTA	209	68.28	1.70	1.161	1.366	1.239	**0.933**	10741.0
66	TTTTTTGTCTGGTGTGGTAA	210	62.37	1.70	0.294	1.366	0.701	**0.950**	9187.5
67	TTTTTGTCTGGTGTGGTAAG	211	62.23	1.70	0.273	1.366	0.689	**0.941**	7871.0
68	TTTTGTCTGGTGTGGTAAGT	212	65.28	1.20	0.721	0.931	0.801	**0.921**	7209.1
69	TTTGTCTGGTGTGGTAAGTC	213	66.56	1.20	0.908	0.931	0.917	**0.959**	8052.3
70	TTGTCTGGTGTGGTAAGTCC	214	70.25	0.30	1.449	0.149	0.955	**1.022**	7230.6
71	TGTCTGGTGTGGTAAGTCCC	215	73.77	-0.10	1.966	-0.199	1.143	**0.998**	6809.5
72	GTCTGGTGTGGTAAGTCCCC	216	77.74	-0.10	2.549	-0.199	1.504	**0.913**	7442.8
73	TCTGGTGTGGTAAGTCCCCA	217	75.28	-0.50	2.187	-0.547	1.148	~~0.824~~	2627.7
74	CTGGTGTGGTAAGTCCCCAC	218	74.18	-2.10	2.026	-1.938	0.519	~~0.781~~	1315.0
75	TGGTGTGGTAAGTCCCCACC	219	75.80	-3.50	2.263	-3.156	0.204	~~0.680~~	4182.3
76	GGTGTGGTAAGTCCCCACCT	220	77.89	-3.80	2.571	-3.417	0.296	~~0.518~~	474.7
77	GTGTGGTAAGTCCCCACCTC	221	77.05	-2.50	2.448	-2.286	0.649	~~0.429~~	682.4
78	TGTGGTAAGTCCCCACCTCA	222	74.71	-2.50	2.105	-2.286	0.436	~~0.465~~	679.1
79	GTGGTAAGTCCCCACCTCAA	223	72.54	-2.10	1.785	-1.938	0.370	~~0.584~~	924.0
80	TGGTAAGTCCCCACCTCAAC	224	69.94	-0.90	1.404	-0.895	0.531	~~0.667~~	835.5

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
81	GGTAAGTCCCCACCTCAACA	225	71.14	-0.50	1.580	-0.547	0.772	~~0.687~~	1213.6
82	GTAAGTCCCCACCTCAACAG	226	68.97	0.90	1.262	0.670	1.037	~~0.763~~	1106.1
83	TAAGTCCCCACCTCAACAGA	227	67.18	0.90	0.999	0.670	0.874	**0.872**	1009.0
84	AAGTCCCCACCTCAACAGAT	228	67.68	0.50	1.073	0.323	0.788	**0.908**	1656.2
85	AGTCCCCACCTCAACAGATG	229	69.68	0.50	1.366	0.323	0.970	~~0.831~~	2178.3
86	GTCCCCACCTCAACAGATGT	230	72.56	0.20	1.789	0.062	1.132	~~0.679~~	2567.0
87	TCCCCACCTCAACAGATGTT	231	69.77	-0.10	1.379	-0.199	0.779	~~0.522~~	3000.5
88	CCCCACCTCAACAGATGTTG	232	68.19	-1.30	1.148	-1.243	0.240	~~0.354~~	2025.4
89	CCCACCTCAACAGATGTTGT	233	67.78	-2.00	1.087	-1.851	-0.030	~~0.164~~	429.2
90	CCACCTCAACAGATGTTGTC	234	65.65	-2.00	0.775	-1.851	-0.223	~~-0.041~~	157.9
91	CACCTCAACAGATGTTGTCT	235	63.85	-2.00	0.511	-1.851	-0.387	~~-0.244~~	135.3
92	ACCTCAACAGATGTTGTCTC	236	64.11	-2.00	0.549	-1.851	-0.363	~~-0.339~~	330.8
93	CCTCAACAGATGTTGTCTCA	237	64.77	-2.00	0.646	-1.851	-0.303	~~-0.370~~	900.0
94	CTCAACAGATGTTGTCTCAG	238	61.08	-2.00	0.104	-1.851	-0.639	~~-0.300~~	1177.0
95	TCAACAGATGTTGTCTCAGC	239	63.40	-2.00	0.444	-1.851	-0.428	~~-0.117~~	795.1
96	CAACAGATGTTGTCTCAGCT	240	63.91	-1.60	0.520	-1.504	-0.249	~~0.081~~	889.2
97	AACAGATGTTGTCTCAGCTC	241	64.19	-0.10	0.560	-0.199	0.272	~~0.287~~	1703.6
98	ACAGATGTTGTCTCAGCTCC	242	70.61	0.00	1.503	-0.112	0.889	~~0.598~~	3115.2
99	CAGATGTTGTCTCAGCTCCT	243	72.08	0.00	1.719	-0.112	1.023	**0.847**	4445.0
100	AGATGTTGTCTCAGCTCCTC	244	72.66	0.20	1.803	0.062	1.141	**1.070**	6762.8
101	GATGTTGTCTCAGCTCCTCT	245	74.49	0.90	2.071	0.670	1.539	**1.227**	8845.0
102	ATGTTGTCTCAGCTCCTCTA	246	72.38	0.80	1.763	0.583	1.314	**1.253**	9010.6
103	TGTTGTCTCAGCTCCTCTAT	247	72.38	0.80	1.763	0.583	1.314	**1.260**	19941.0
104	GTTGTCTCAGCTCCTCTATT	248	72.97	0.80	1.849	0.583	1.368	**1.257**	12577.0
105	TTGTCTCAGCTCCTCTATTT	249	69.70	0.80	1.369	0.583	1.071	**1.149**	7503.3
106	TGTCTCAGCTCCTCTATTTT	250	69.70	0.80	1.369	0.583	1.071	**1.098**	7033.8
107	GTCTCAGCTCCTCTATTTTT	251	70.26	0.80	1.451	0.583	1.121	**1.024**	8276.7

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
108	TCTCAGCTCCTCTATTTTTG	252	66.57	0.80	0.910	0.583	0.786	**0.942**	2899.0
109	CTCAGCTCCTCTATTTTTGT	253	68.39	0.80	1.177	0.583	0.952	**0.923**	2935.0
110	TCAGCTCCTCTATTTTTGTT	254	66.69	0.80	0.927	0.583	0.796	**0.930**	1512.8
111	CAGCTCCTCTATTTTTGTTC	255	66.69	0.80	0.927	0.583	0.796	**0.872**	1708.8
112	AGCTCCTCTATTTTTGTTCT	256	67.52	1.00	1.050	0.757	0.939	**0.833**	1977.3
113	GCTCCTCTATTTTTGTTCTA	257	66.63	1.80	0.919	1.453	1.122	~~0.809~~	2114.8
114	CTCCTCTATTTTTGTTCTAT	258	62.13	1.80	0.259	1.453	0.713	~~0.766~~	1527.3
115	TCCTCTATTTTTGTTCTATG	259	59.97	1.80	-0.058	1.453	0.516	~~0.695~~	1536.8
116	CCTCTATTTTTGTTCTATGC	260	62.84	1.80	0.363	1.453	0.777	~~0.642~~	1824.5
117	CTCTATTTTTGTTCTATGCT	261	60.87	1.50	0.074	1.192	0.499	~~0.588~~	1169.2
118	TCTATTTTTGTTCTATGCTG	262	58.71	1.50	-0.244	1.192	0.302	~~0.649~~	683.7
119	CTATTTTTGTTCTATGCTGC	263	61.60	1.50	0.181	1.192	0.565	~~0.765~~	1306.8
120	TATTTTTGTTCTATGCTGCC	264	63.53	1.50	0.464	1.192	0.741	~~0.831~~	2523.6
121	ATTTTTGTTCTATGCTGCCC	265	67.96	1.50	1.113	1.192	1.143	**0.931**	6682.0
122	TTTTTGTTCTATGCTGCCCT	266	69.96	1.50	1.407	1.192	1.325	**1.060**	9417.4
123	TTTTGTTCTATGCTGCCCTA	267	69.01	1.50	1.267	1.192	1.239	**1.151**	10339.0
124	TTTGTTCTATGCTGCCCTAT	268	68.62	1.50	1.210	1.192	1.203	**1.254**	10750.0
125	TTGTTCTATGCTGCCCTATT	269	68.62	1.50	1.210	1.192	1.203	**1.282**	11180.0
126	TGTTCTATGCTGCCCTATTT	270	68.62	1.50	1.210	1.192	1.203	**1.271**	11060.0
127	GTTCTATGCTGCCCTATTTC	271	70.37	1.80	1.468	1.453	1.462	**1.221**	16074.0
128	TTCTATGCTGCCCTATTTCT	272	69.00	1.80	1.266	1.453	1.337	**1.144**	9183.8
129	TCTATGCTGCCCTATTTCTA	273	68.05	1.80	1.127	1.453	1.251	**1.082**	8617.8
130	CTATGCTGCCCTATTTCTAA	274	64.38	1.70	0.589	1.366	0.884	**1.040**	7286.8
131	TATGCTGCCCTATTTCTAAG	275	62.71	1.50	0.344	1.192	0.666	**0.978**	3642.4
132	ATGCTGCCCTATTTCTAAGT	276	66.39	0.80	0.883	0.583	0.769	**0.883**	3799.7
133	TGCTGCCCTATTTCTAAGTC	277	67.95	0.80	1.112	0.583	0.911	~~0.749~~	3408.3
134	GCTGCCCTATTTCTAAGTCA	278	69.25	0.80	1.303	0.583	1.030	~~0.644~~	4017.4

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
135	CTGCCCTATTTCTAAGTCAG	279	65.26	0.80	0.718	0.583	0.667	~~0.536~~	2197.2
136	TGCCCTATTTCTAAGTCAGA	280	64.63	-0.10	0.626	-0.199	0.312	~~0.412~~	1125.0
137	GCCCTATTTCTAAGTCAGAT	281	64.73	-0.60	0.639	-0.634	0.156	~~0.244~~	1306.3
138	CCCTATTTCTAAGTCAGATC	282	61.98	-0.60	0.236	-0.634	-0.094	~~0.024~~	1019.5
139	CCTATTTCTAAGTCAGATCC	283	61.98	-0.60	0.236	-0.634	-0.094	~~-0.129~~	1852.3
140	CTATTTCTAAGTCAGATCCT	284	60.05	-0.60	-0.046	-0.634	-0.270	~~-0.214~~	3159.3
141	TATTTCTAAGTCAGATCCTA	285	57.43	-0.60	-0.430	-0.634	-0.508	~~-0.281~~	2604.8
142	ATTTCTAAGTCAGATCCTAC	286	58.59	-0.60	-0.261	-0.634	-0.402	~~-0.315~~	3986.1
143	TTTCTAAGTCAGATCCTACA	287	59.91	-0.60	-0.068	-0.634	-0.283	~~-0.285~~	4500.7
144	TTCTAAGTCAGATCCTACAT	288	59.55	-0.60	-0.120	-0.634	-0.315	~~-0.233~~	4754.5
145	TCTAAGTCAGATCCTACATA	289	58.62	-0.40	-0.257	-0.460	-0.334	~~-0.165~~	3802.1
146	CTAAGTCAGATCCTACATAC	290	57.80	1.20	-0.377	0.931	0.120	~~-0.111~~	5069.4
147	TAAGTCAGATCCTACATACA	291	57.13	1.30	-0.476	1.018	0.092	~~-0.059~~	3965.2
148	AAGTCAGATCCTACATACAA	292	55.78	1.30	-0.673	1.018	-0.030	~~-0.031~~	3862.3
149	AGTCAGATCCTACATACAAA	293	55.78	1.30	-0.673	1.018	-0.030	~~-0.020~~	2868.9
150	GTCAGATCCTACATACAAAT	294	55.62	1.70	-0.697	1.366	0.087	~~-0.089~~	3542.9
151	TCAGATCCTACATACAAATC	295	54.02	1.50	-0.932	1.192	-0.125	~~-0.122~~	2477.1
152	CAGATCCTACATACAAATCA	296	54.07	1.10	-0.924	0.844	-0.252	~~-0.091~~	2522.4
153	AGATCCTACATACAAATCAT	297	52.83	1.10	-1.106	0.844	-0.365	~~-0.045~~	2554.6
154	GATCCTACATACAAATCATC	298	53.87	1.50	-0.953	1.192	-0.138	~~-0.031~~	3580.0
155	ATCCTACATACAAATCATCC	299	56.33	1.80	-0.591	1.453	0.185	~~-0.067~~	5937.7
156	TCCTACATACAAATCATCCA	300	57.54	1.80	-0.415	1.453	0.295	~~-0.111~~	4606.7
157	CCTACATACAAATCATCCAT	301	56.32	1.80	-0.594	1.453	0.184	~~-0.159~~	4877.2
158	CTACATACAAATCATCCATG	302	52.68	1.10	-1.128	0.844	-0.379	~~-0.278~~	2608.6
159	TACATACAAATCATCCATGT	303	53.56	0.30	-0.999	0.149	-0.563	~~-0.469~~	1491.7
160	ACATACAAATCATCCATGTA	304	53.56	-0.10	-0.999	-0.199	-0.695	~~-0.644~~	1364.3
161	CATACAAATCATCCATGTAT	305	53.07	-0.80	-1.071	-0.808	-0.971	*-0.751*	1089.8

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
162	ATACAAATCATCCATGTATT	306	52.11	-1.10	-1.211	-1.069	-1.157	*-0.818*	1008.6
163	TACAAATCATCCATGTATTG	307	52.08	-0.40	-1.215	-0.460	-0.928	*-0.891*	624.8
164	ACAAATCATCCATGTATTGA	308	53.86	0.20	-0.955	0.062	-0.568	*-0.921*	535.8
165	CAAATCATCCATGTATTGAT	309	53.36	-0.50	-1.027	-0.547	-0.845	*-0.860*	3019.6
166	AAATCATCCATGTATTGATA	310	51.57	-0.70	-1.291	-0.721	-1.074	*-0.753*	214.0
167	AATCATCCATGTATTGATAG	311	53.47	-0.70	-1.012	-0.721	-0.901	~~*-0.685*~~	212.7
168	ATCATCCATGTATTGATAGA	312	56.66	-0.50	-0.543	-0.547	-0.545	~~*-0.709*~~	165.2
169	TCATCCATGTATTGATAGAT	313	56.66	-0.10	-0.543	-0.199	-0.412	~~*-0.686*~~	166.0
170	CATCCATGTATTGATAGATA	314	54.80	0.30	-0.817	0.149	-0.450	~~*-0.622*~~	151.0
171	ATCCATGTATTGATAGATAA	315	51.69	0.30	-1.273	0.149	-0.733	~~*-0.621*~~	101.8
172	TCCATGTATTGATAGATAAC	316	52.19	0.30	-1.199	0.149	-0.687	~~*-0.721*~~	84.0
173	CCATGTATTGATAGATAACT	317	52.89	0.30	-1.097	0.149	-0.623	*-0.850*	130.3
174	CATGTATTGATAGATAACTA	318	48.47	0.70	-1.746	0.496	-0.894	*-0.937*	67.8
175	ATGTATTGATAGATAACTAT	319	47.12	0.00	-1.944	-0.112	-1.248	*-1.006*	65.7
176	TGTATTGATAGATAACTATG	320	47.11	-0.20	-1.945	-0.286	-1.315	*-1.048*	90.0
177	GTATTGATAGATAACTATGT	321	49.90	-0.20	-1.536	-0.286	-1.061	*-1.099*	125.9
178	TATTGATAGATAACTATGTC	322	48.24	-0.20	-1.779	-0.286	-1.212	*-1.083*	132.6
179	ATTGATAGATAACTATGTCT	323	50.78	-0.20	-1.407	-0.286	-0.981	*-0.998*	167.4
180	TTGATAGATAACTATGTCTG	324	50.75	-0.20	-1.411	-0.286	-0.984	*-0.916*	219.0
181	TGATAGATAACTATGTCTGG	325	53.01	-0.20	-1.080	-0.286	-0.778	*-0.866*	722.6
182	GATAGATAACTATGTCTGGA	326	54.36	-0.20	-0.881	-0.286	-0.655	*-0.774*	825.1
183	ATAGATAACTATGTCTGGAT	327	53.04	-0.10	-1.074	-0.199	-0.742	~~*-0.679*~~	844.4
184	TAGATAACTATGTCTGGATT	328	53.37	-0.10	-1.027	-0.199	-0.712	~~*-0.569*~~	912.6
185	AGATAACTATGTCTGGATTT	329	54.27	0.10	-0.895	-0.025	-0.565	~~*-0.449*~~	1301.8
186	GATAACTATGTCTGGATTTT	330	54.43	0.80	-0.870	0.583	-0.318	~~*-0.335*~~	1367.4
187	ATAACTATGTCTGGATTTTG	331	53.08	1.50	-1.070	1.192	-0.210	~~*-0.177*~~	1284.2
188	TAACTATGTCTGGATTTTGT	332	56.05	1.50	-0.634	1.192	0.060	~~*-0.026*~~	1162.5

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
189	AACTATGTCTGGATTTTGTT	333	56.97	1.50	-0.499	1.192	0.144	~~0.081~~	1396.7
190	ACTATGTCTGGATTTTGTTT	334	59.38	1.50	-0.145	1.192	0.363	~~0.176~~	1348.3
191	CTATGTCTGGATTTTGTTTT	335	59.16	1.50	-0.177	1.192	0.343	~~0.261~~	1092.8
192	TATGTCTGGATTTTGTTTTT	336	57.45	1.50	-0.428	1.192	0.188	~~0.234~~	912.6
193	ATGTCTGGATTTTGTTTTTT	337	58.41	1.70	-0.287	1.366	0.341	~~0.123~~	994.3
194	TGTCTGGATTTTGTTTTTTA	338	57.81	2.00	-0.375	1.627	0.386	~~-0.079~~	840.7
195	GTCTGGATTTTGTTTTTTAA	339	55.82	1.00	-0.667	0.757	-0.126	~~-0.311~~	941.9
196	TCTGGATTTTGTTTTTTAAA	340	50.98	0.80	-1.377	0.583	-0.632	~~-0.488~~	84.9
197	CTGGATTTTGTTTTTTAAAA	341	48.16	0.30	-1.790	0.149	-1.054	~~-0.670~~	78.6
198	TGGATTTTGTTTTTTAAAAG	342	46.41	0.10	-2.048	-0.025	-1.279	*-0.851*	93.2
199	GGATTTTGTTTTTTAAAAGG	343	48.87	0.10	-1.686	-0.025	-1.055	*-0.933*	56.0
200	GATTTTGTTTTTTAAAAGGC	344	50.22	0.10	-1.488	-0.025	-0.932	*-0.912*	49.9
201	ATTTTGTTTTTTAAAAGGCT	345	50.84	0.10	-1.397	-0.025	-0.876	*-0.843*	55.0
202	TTTTGTTTTTTAAAAGGCTC	346	52.03	0.30	-1.223	0.149	-0.702	*-0.768*	64.6
203	TTTGTTTTTTAAAAGGCTCT	347	53.64	0.50	-0.987	0.323	-0.489	~~-0.724~~	162.8
204	TTGTTTTTTAAAAGGCTCTA	348	52.76	0.50	-1.115	0.323	-0.569	~~-0.706~~	265.8
205	TGTTTTTTAAAAGGCTCTAA	349	50.71	0.50	-1.417	0.323	-0.756	~~-0.677~~	288.5
206	GTTTTTTAAAAGGCTCTAAG	350	50.86	0.50	-1.395	0.323	-0.742	~~-0.672~~	548.4
207	TTTTTTAAAAGGCTCTAAGA	351	49.40	0.70	-1.609	0.496	-0.809	~~-0.698~~	524.7
208	TTTTTAAAAGGCTCTAAGAT	352	49.11	1.20	-1.651	0.931	-0.670	*-0.746*	937.9
209	TTTTAAAAGGCTCTAAGATT	353	49.11	1.20	-1.651	0.931	-0.670	*-0.790*	1440.3
210	TTTAAAAGGCTCTAAGATTT	354	49.11	1.20	-1.651	0.931	-0.670	*-0.820*	1633.3
211	TTAAAAGGCTCTAAGATTTT	355	49.11	0.50	-1.651	0.323	-0.901	*-0.735*	1987.4
212	TAAAAGGCTCTAAGATTTTT	356	49.11	0.00	-1.651	-0.112	-1.067	~~-0.627~~	1792.3
213	AAAAGGCTCTAAGATTTTTG	357	49.63	0.20	-1.575	0.062	-0.953	~~-0.495~~	2218.9
214	AAAGGCTCTAAGATTTTTGT	358	54.13	1.20	-0.914	0.931	-0.213	~~-0.365~~	2371.4
215	AAGGCTCTAAGATTTTTGTC	359	57.38	1.20	-0.439	0.931	0.082	~~-0.238~~	3308.9

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
216	AGGCTCTAAGATTTTTGTCA	360	60.78	0.80	0.061	0.583	0.260	~~-0.087~~	4070.5
217	GGCTCTAAGATTTTTGTCAT	361	60.56	0.80	0.028	0.583	0.239	~~0.048~~	5394.5
218	GCTCTAAGATTTTTGTCATG	362	57.81	0.80	-0.376	0.583	-0.011	~~0.051~~	2025.5
219	CTCTAAGATTTTTGTCATGC	363	57.81	0.80	-0.376	0.583	-0.011	~~-0.006~~	1741.9
220	TCTAAGATTTTTGTCATGCT	364	57.81	0.80	-0.376	0.583	-0.011	~~-0.065~~	1707.6
221	CTAAGATTTTTGTCATGCTA	365	55.87	0.80	-0.660	0.583	-0.187	~~-0.089~~	1783.0
222	TAAGATTTTTGTCATGCTAC	366	54.43	0.80	-0.872	0.583	-0.319	~~-0.076~~	3131.4
223	AAGATTTTTGTCATGCTACT	367	56.99	0.60	-0.495	0.410	-0.151	~~-0.082~~	4892.5
224	AGATTTTTGTCATGCTACTT	368	59.39	0.60	-0.144	0.410	0.067	~~-0.053~~	5856.4
225	GATTTTTGTCATGCTACTTT	369	59.54	0.60	-0.122	0.410	0.080	~~0.015~~	6439.0
226	ATTTTTGTCATGCTACTTTG	370	58.09	0.60	-0.334	0.410	-0.051	~~0.069~~	5820.3
227	TTTTTGTCATGCTACTTTGG	371	60.78	0.60	0.060	0.410	0.193	~~0.095~~	5189.6
228	TTTTGTCATGCTACTTTGGA	372	61.79	0.60	0.209	0.410	0.285	~~0.079~~	4721.7
229	TTTGTCATGCTACTTTGGAA	373	59.35	0.60	-0.149	0.410	0.063	~~0.075~~	4221.0
230	TTGTCATGCTACTTTGGAAT	374	59.00	0.60	-0.200	0.410	0.032	~~0.056~~	4279.0
231	TGTCATGCTACTTTGGAATA	375	58.10	0.60	-0.333	0.410	-0.051	~~0.004~~	4102.0
232	GTCATGCTACTTTGGAATAT	376	58.16	0.90	-0.324	0.670	0.054	~~-0.022~~	5069.8
233	TCATGCTACTTTGGAATATT	377	55.52	0.90	-0.711	0.670	-0.186	~~-0.015~~	2407.9
234	CATGCTACTTTGGAATATTG	378	54.23	1.30	-0.900	1.018	-0.171	~~0.016~~	2443.0
235	ATGCTACTTTGGAATATTGC	379	56.90	1.40	-0.508	1.105	0.105	~~0.058~~	2324.3
236	TGCTACTTTGGAATATTGCT	380	58.82	0.90	-0.227	0.670	0.114	~~0.099~~	1894.1
237	GCTACTTTGGAATATTGCTG	381	58.82	1.30	-0.227	1.018	0.246	~~0.180~~	2363.8
238	CTACTTTGGAATATTGCTGG	382	57.35	1.70	-0.443	1.366	0.244	~~0.270~~	1363.0
239	TACTTTGGAATATTGCTGGT	383	58.39	1.70	-0.290	1.366	0.339	~~0.299~~	1217.5
240	ACTTTGGAATATTGCTGGTG	384	58.88	1.70	-0.217	1.366	0.384	~~0.340~~	1621.8
241	CTTTGGAATATTGCTGGTGA	385	59.64	1.70	-0.106	1.366	0.453	~~0.346~~	1438.2
242	TTTGGAATATTGCTGGTGAT	386	57.72	1.80	-0.388	1.453	0.311	~~0.345~~	1608.0

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
243	TTGGAATATTGCTGGTGATC	387	58.73	1.80	-0.241	1.453	0.403	~~0.302~~	2334.6
244	TGGAATATTGCTGGTGATCC	388	62.18	0.50	0.266	0.323	0.288	~~0.241~~	3776.7
245	GGAATATTGCTGGTGATCCT	389	64.19	-0.20	0.561	-0.286	0.239	~~0.216~~	5648.7
246	GAATATTGCTGGTGATCCTT	390	61.99	-0.20	0.238	-0.286	0.039	~~0.261~~	5358.8
247	AATATTGCTGGTGATCCTTT	391	61.03	-0.20	0.097	-0.286	-0.049	~~0.316~~	5517.2
248	ATATTGCTGGTGATCCTTTC	392	64.63	-0.20	0.625	-0.286	0.279	~~0.368~~	6246.4
249	TATTGCTGGTGATCCTTTCC	393	68.48	-0.20	1.190	-0.286	0.629	~~0.444~~	9975.1
250	ATTGCTGGTGATCCTTTCCA	394	70.22	-0.20	1.446	-0.286	0.788	~~0.599~~	11990.0
251	TTGCTGGTGATCCTTTCCAT	395	70.22	-0.60	1.446	-0.634	0.655	~~0.756~~	11543.0
252	TGCTGGTGATCCTTTCCATC	396	71.48	-0.60	1.631	-0.634	0.770	**0.862**	14125.0
253	GCTGGTGATCCTTTCCATCC	397	75.32	-0.60	2.193	-0.634	1.119	**0.936**	23489.0
254	CTGGTGATCCTTTCCATCCC	398	74.58	-0.60	2.085	-0.634	1.052	**1.022**	15975.0
255	TGGTGATCCTTTCCATCCCT	399	74.58	-0.70	2.085	-0.721	1.019	**1.082**	16053.0
256	GGTGATCCTTTCCATCCCTG	400	74.58	-0.30	2.085	-0.373	1.151	**1.136**	19205.0
257	GTGATCCTTTCCATCCCTGT	401	75.40	0.20	2.206	0.062	1.391	**1.080**	17872.0
258	TGATCCTTTCCATCCCTGTG	402	71.89	0.20	1.691	0.062	1.072	**0.955**	12871.0
259	GATCCTTTCCATCCCTGTGG	403	74.58	-0.30	2.085	-0.373	1.151	~~0.809~~	8792.7
260	ATCCTTTCCATCCCTGTGGA	404	74.58	-1.60	2.085	-1.504	0.721	~~0.653~~	5609.6
261	TCCTTTCCATCCCTGTGGAA	405	72.27	-2.60	1.746	-2.373	0.181	~~0.451~~	3018.0
262	CCTTTCCATCCCTGTGGAAG	406	71.00	-2.80	1.559	-2.547	-0.001	~~0.308~~	1802.6
263	CTTTCCATCCCTGTGGAAGC	407	71.60	-2.80	1.648	-2.547	0.054	~~0.205~~	1074.0
264	TTTCCATCCCTGTGGAAGCA	408	70.81	-2.80	1.532	-2.547	-0.018	~~0.120~~	1132.5
265	TTCCATCCCTGTGGAAGCAC	409	71.02	-2.60	1.562	-2.373	0.067	~~0.071~~	1454.5
266	TCCATCCCTGTGGAAGCACA	410	71.74	-1.70	1.669	-1.591	0.430	~~0.032~~	1676.8
267	CCATCCCTGTGGAAGCACAT	411	70.20	-2.20	1.443	-2.025	0.125	~~0.026~~	2268.9
268	CATCCCTGTGGAAGCACATT	412	67.07	-2.20	0.983	-2.025	-0.160	~~0.004~~	1682.6
269	ATCCCTGTGGAAGCACATTG	413	65.82	-2.20	0.801	-2.025	-0.273	~~-0.070~~	1753.9

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
270	TCCCTGTGGAAGCACATTGT	414	68.98	-2.20	1.263	-2.025	0.014	~~-0.220~~	1281.8
271	CCCTGTGGAAGCACATTGTA	415	66.92	-2.20	0.962	-2.025	-0.173	~~-0.344~~	1227.8
272	CCTGTGGAAGCACATTGTAC	416	63.84	-2.20	0.509	-2.025	-0.454	~~-0.337~~	700.3
273	CTGTGGAAGCACATTGTACT	417	62.01	-2.20	0.241	-2.025	-0.620	~~-0.307~~	618.7
274	TGTGGAAGCACATTGTACTG	418	59.99	-2.00	-0.056	-1.851	-0.738	~~-0.324~~	771.5
275	GTGGAAGCACATTGTACTGA	419	61.39	-0.50	0.149	-0.547	-0.115	~~-0.347~~	1180.6
276	TGGAAGCACATTGTACTGAT	420	58.35	0.50	-0.296	0.323	-0.061	~~-0.331~~	1160.5
277	GGAAGCACATTGTACTGATA	421	57.86	0.50	-0.368	0.323	-0.106	~~-0.239~~	1314.7
278	GAAGCACATTGTACTGATAT	422	55.32	0.50	-0.740	0.323	-0.336	~~-0.141~~	1102.5
279	AAGCACATTGTACTGATATC	423	55.30	0.50	-0.744	0.323	-0.339	~~-0.209~~	1222.1
280	AGCACATTGTACTGATATCT	424	59.26	0.50	-0.162	0.323	0.022	~~-0.302~~	1893.2
281	GCACATTGTACTGATATCTA	425	58.48	0.50	-0.277	0.323	-0.049	~~-0.398~~	2097.7
282	CACATTGTACTGATATCTAA	426	52.51	0.50	-1.152	0.323	-0.592	~~-0.446~~	1237.8
283	ACATTGTACTGATATCTAAT	427	51.20	0.50	-1.345	0.323	-0.711	~~-0.443~~	959.5
284	CATTGTACTGATATCTAATC	428	51.89	0.10	-1.244	-0.025	-0.781	~~-0.472~~	1149.1
285	ATTGTACTGATATCTAATCC	429	54.53	-0.30	-0.856	-0.373	-0.672	~~-0.490~~	2351.3
286	TTGTACTGATATCTAATCCC	430	58.41	-0.30	-0.287	-0.373	-0.320	~~-0.436~~	4191.6
287	TGTACTGATATCTAATCCCT	431	59.99	-0.30	-0.055	-0.373	-0.176	~~-0.320~~	5565.8
288	GTACTGATATCTAATCCCTG	432	59.99	-0.30	-0.055	-0.373	-0.176	~~-0.202~~	9980.2
289	TACTGATATCTAATCCCTGG	433	59.52	-0.30	-0.124	-0.373	-0.218	~~-0.084~~	6318.9
290	ACTGATATCTAATCCCTGGT	434	63.07	-0.30	0.397	-0.373	0.104	~~0.023~~	7749.5
291	CTGATATCTAATCCCTGGTG	435	62.43	-0.30	0.303	-0.373	0.046	~~0.184~~	8165.3
292	TGATATCTAATCCCTGGTGT	436	63.60	-0.30	0.474	-0.373	0.152	~~0.365~~	9107.6
293	GATATCTAATCCCTGGTGTC	437	65.19	0.10	0.707	-0.025	0.429	~~0.566~~	13914.0
294	ATATCTAATCCCTGGTGTCT	438	65.82	1.50	0.800	1.192	0.949	~~0.698~~	15093.0
295	TATCTAATCCCTGGTGTCTC	439	67.41	1.50	1.033	1.192	1.093	~~0.822~~	18647.0
296	ATCTAATCCCTGGTGTCTCA	440	69.20	1.30	1.296	1.018	1.190	**0.904**	21810.0

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
297	TCTAATCCCTGGTGTCTCAT	441	69.20	0.80	1.296	0.583	1.025	**0.996**	20102.0
298	CTAATCCCTGGTGTCTCATT	442	67.98	0.80	1.117	0.583	0.914	**1.052**	20967.0
299	TAATCCCTGGTGTCTCATTG	443	65.90	0.80	0.811	0.583	0.725	**1.092**	18200.0
300	AATCCCTGGTGTCTCATTGT	444	69.78	0.80	1.380	0.583	1.077	**1.088**	19845.0
301	ATCCCTGGTGTCTCATTGTT	445	72.61	0.80	1.797	0.583	1.336	**1.057**	19231.0
302	TCCCTGGTGTCTCATTGTTT	446	73.04	0.80	1.860	0.583	1.375	**0.981**	17629.0
303	CCCTGGTGTCTCATTGTTTA	447	70.72	0.80	1.519	0.583	1.164	**0.918**	17009.0
304	CCTGGTGTCTCATTGTTTAT	448	66.82	0.80	0.946	0.583	0.808	~~0.800~~	11580.0
305	CTGGTGTCTCATTGTTTATA	449	62.17	0.80	0.264	0.583	0.386	~~0.600~~	8374.6
306	TGGTGTCTCATTGTTTATAC	450	60.65	0.90	0.042	0.670	0.281	~~0.355~~	6153.3
307	GGTGTCTCATTGTTTATACT	451	62.88	0.20	0.369	0.062	0.252	~~0.177~~	7134.0
308	GTGTCTCATTGTTTATACTA	452	59.43	0.20	-0.138	0.062	-0.062	~~0.050~~	4435.2
309	TGTCTCATTGTTTATACTAG	453	56.35	0.20	-0.589	0.062	-0.342	~~-0.043~~	2035.5
310	GTCTCATTGTTTATACTAGG	454	59.21	0.20	-0.170	0.062	-0.082	~~-0.149~~	2466.6
311	TCTCATTGTTTATACTAGGT	455	59.21	0.20	-0.170	0.062	-0.082	~~-0.268~~	1080.9
312	CTCATTGTTTATACTAGGTA	456	57.15	0.20	-0.472	0.062	-0.269	~~-0.325~~	956.0
313	TCATTGTTTATACTAGGTAT	457	55.08	0.20	-0.776	0.062	-0.458	~~-0.302~~	529.4
314	CATTGTTTATACTAGGTATG	458	53.70	0.20	-0.978	0.062	-0.583	~~-0.328~~	471.4
315	ATTGTTTATACTAGGTATGG	459	55.01	0.20	-0.785	0.062	-0.463	~~-0.389~~	510.4
316	TTGTTTATACTAGGTATGGT	460	58.17	0.20	-0.322	0.062	-0.176	~~-0.486~~	531.0
317	TGTTTATACTAGGTATGGTA	461	57.21	0.20	-0.463	0.062	-0.264	~~-0.560~~	613.3
318	GTTTATACTAGGTATGGTAA	462	55.23	0.00	-0.753	-0.112	-0.510	~~-0.620~~	685.1
319	TTTATACTAGGTATGGTAAA	463	50.42	0.00	-1.459	-0.112	-0.947	~~-0.639~~	300.0
320	TTATACTAGGTATGGTAAAT	464	50.12	0.00	-1.504	-0.112	-0.975	~~-0.672~~	316.1
321	TATACTAGGTATGGTAAATG	465	49.79	0.00	-1.551	-0.112	-1.004	~~-0.655~~	387.5
322	ATACTAGGTATGGTAAATGC	466	54.30	0.00	-0.889	-0.112	-0.594	~~-0.557~~	685.7
323	TACTAGGTATGGTAAATGCA	467	55.59	0.20	-0.700	0.062	-0.411	~~-0.430~~	759.6

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
324	ACTAGGTATGGTAAATGCAG	468	56.32	0.80	-0.593	0.583	-0.146	~~-0.291~~	1050.2
325	CTAGGTATGGTAAATGCAGT	469	58.78	1.10	-0.232	0.844	0.177	~~-0.157~~	1020.4
326	TAGGTATGGTAAATGCAGTA	470	56.24	1.10	-0.605	0.844	-0.054	~~-0.109~~	742.6
327	AGGTATGGTAAATGCAGTAT	471	56.81	1.10	-0.521	0.844	-0.002	~~-0.132~~	889.6
328	GGTATGGTAAATGCAGTATA	472	56.07	1.10	-0.631	0.844	-0.070	~~-0.182~~	858.8
329	GTATGGTAAATGCAGTATAC	473	54.02	1.10	-0.931	0.844	-0.256	~~-0.262~~	379.0
330	TATGGTAAATGCAGTATACT	474	53.06	0.40	-1.071	0.236	-0.575	~~-0.257~~	166.7
331	ATGGTAAATGCAGTATACTT	475	53.94	0.40	-0.943	0.236	-0.495	~~-0.249~~	215.3
332	TGGTAAATGCAGTATACTTC	476	55.21	0.40	-0.757	0.236	-0.380	~~-0.303~~	103.2
333	GGTAAATGCAGTATACTTCC	477	59.15	0.40	-0.178	0.236	-0.021	~~-0.326~~	246.3
334	GTAAATGCAGTATACTTCCT	478	58.53	0.80	-0.269	0.583	0.055	~~-0.303~~	163.4
335	TAAATGCAGTATACTTCCTG	479	55.54	0.10	-0.708	-0.025	-0.448	~~-0.264~~	294.1
336	AAATGCAGTATACTTCCTGA	480	57.36	-0.30	-0.441	-0.373	-0.415	~~-0.229~~	531.4
337	AATGCAGTATACTTCCTGAA	481	57.36	-0.30	-0.441	-0.373	-0.415	~~-0.233~~	1995.5
338	ATGCAGTATACTTCCTGAAG	482	59.50	-0.30	-0.128	-0.373	-0.221	~~-0.279~~	510.1
339	TGCAGTATACTTCCTGAAGT	483	62.63	-0.90	0.332	-0.895	-0.134	~~-0.264~~	555.4
340	GCAGTATACTTCCTGAAGTC	484	64.24	-1.10	0.568	-1.069	-0.054	~~-0.238~~	1214.0
341	CAGTATACTTCCTGAAGTCT	485	61.94	-1.10	0.230	-1.069	-0.263	~~-0.237~~	825.7
342	AGTATACTTCCTGAAGTCTT	486	61.00	-1.10	0.094	-1.069	-0.348	~~-0.261~~	1582.6
343	GTATACTTCCTGAAGTCTTC	487	62.28	-1.10	0.281	-1.069	-0.232	~~-0.278~~	2391.8
344	TATACTTCCTGAAGTCTTCA	488	60.34	-1.10	-0.004	-1.069	-0.409	~~-0.273~~	2276.3
345	ATACTTCCTGAAGTCTTCAT	489	60.91	-1.20	0.080	-1.156	-0.389	~~-0.252~~	2702.8
346	TACTTCCTGAAGTCTTCATC	490	62.40	-1.20	0.299	-1.156	-0.254	~~-0.274~~	3781.7
347	ACTTCCTGAAGTCTTCATCT	491	65.05	-1.20	0.686	-1.156	-0.014	~~-0.314~~	5343.4
348	CTTCCTGAAGTCTTCATCTA	492	63.86	-1.20	0.512	-1.156	-0.122	~~-0.314~~	6309.0
349	TTCCTGAAGTCTTCATCTAA	493	59.70	-1.20	-0.098	-1.156	-0.500	~~-0.332~~	6372.4
350	TCCTGAAGTCTTCATCTAAG	494	59.55	-1.20	-0.120	-1.156	-0.513	~~-0.369~~	3835.3

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
351	CCTGAAGTCTTCATCTAAGG	495	60.76	-1.20	0.057	-1.156	-0.404	~~-0.423~~	8925.5
352	CTGAAGTCTTCATCTAAGGG	496	59.48	-1.20	-0.130	-1.156	-0.520	~~-0.472~~	1211.8
353	TGAAGTCTTCATCTAAGGGA	497	58.84	-1.00	-0.224	-0.982	-0.512	~~-0.414~~	609.4
354	GAAGTCTTCATCTAAGGGAA	498	56.91	-0.10	-0.507	-0.199	-0.390	~~-0.358~~	629.1
355	AAGTCTTCATCTAAGGGAAC	499	56.13	-0.10	-0.622	-0.199	-0.461	~~-0.341~~	749.3
356	AGTCTTCATCTAAGGGAACT	500	60.12	-0.10	-0.036	-0.199	-0.098	~~-0.371~~	805.6
357	GTCTTCATCTAAGGGAACTG	501	59.84	-0.10	-0.077	-0.199	-0.124	~~-0.449~~	817.0
358	TCTTCATCTAAGGGAACTGA	502	58.11	-0.10	-0.331	-0.199	-0.281	~~-0.536~~	327.1
359	CTTCATCTAAGGGAACTGAA	503	54.95	-0.60	-0.794	-0.634	-0.733	~~-0.645~~	320.0
360	TTCATCTAAGGGAACTGAAA	504	51.39	-0.60	-1.316	-0.634	-1.057	*-0.822*	84.1
361	TCATCTAAGGGAACTGAAAA	505	49.50	0.10	-1.595	-0.025	-0.998	*-1.002*	67.7
362	CATCTAAGGGAACTGAAAAA	506	46.98	0.10	-1.963	-0.025	-1.227	*-1.171*	62.2
363	ATCTAAGGGAACTGAAAAAT	507	45.78	0.10	-2.140	-0.025	-1.336	*-1.298*	78.9
364	TCTAAGGGAACTGAAAAATA	508	45.27	0.10	-2.214	-0.025	-1.382	*-1.328*	43.2
365	CTAAGGGAACTGAAAAATAT	509	44.36	0.10	-2.349	-0.025	-1.466	*-1.322*	50.4
366	TAAGGGAACTGAAAAATATG	510	42.71	0.10	-2.591	-0.025	-1.616	*-1.242*	43.7
367	AAGGGAACTGAAAAATATGC	511	46.54	0.10	-2.028	-0.025	-1.267	*-1.163*	45.6
368	AGGGAACTGAAAAATATGCA	512	49.21	0.30	-1.637	0.149	-0.958	*-1.119*	49.8
369	GGGAACTGAAAAATATGCAT	513	49.11	1.20	-1.651	0.931	-0.670	*-1.082*	53.2
370	GGAACTGAAAAATATGCATC	514	47.87	1.20	-1.834	0.931	-0.783	*-0.958*	56.6
371	GAACTGAAAAATATGCATCA	515	46.82	0.60	-1.987	0.410	-1.076	*-0.844*	45.3
372	AACTGAAAAATATGCATCAC	516	46.12	0.40	-2.090	0.236	-1.206	*-0.773*	56.3
373	ACTGAAAAATATGCATCACC	517	51.18	0.40	-1.347	0.236	-0.746	~~-0.702~~	61.7
374	CTGAAAAATATGCATCACCC	518	54.20	0.40	-0.905	0.236	-0.471	~~-0.616~~	224.5
375	TGAAAAATATGCATCACCCA	519	53.65	0.60	-0.985	0.410	-0.455	~~-0.476~~	413.0
376	GAAAAATATGCATCACCCAC	520	54.14	1.30	-0.913	1.018	-0.179	~~-0.289~~	1584.0
377	AAAAATATGCATCACCCACA	521	54.14	1.30	-0.913	1.018	-0.179	~~-0.097~~	1846.7

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
378	AAAATATGCATCACCCACAT	522	55.78	1.10	-0.673	0.844	-0.096	~~0.096~~	2445.8
379	AAATATGCATCACCCACATC	523	58.72	0.90	-0.241	0.670	0.105	~~0.291~~	3709.4
380	AATATGCATCACCCACATCC	524	64.13	0.90	0.552	0.670	0.597	~~0.494~~	4548.4
381	ATATGCATCACCCACATCCA	525	67.27	0.90	1.013	0.670	0.883	~~0.680~~	5254.1
382	TATGCATCACCCACATCCAG	526	67.53	0.90	1.051	0.670	0.906	**0.864**	5527.2
383	ATGCATCACCCACATCCAGT	527	71.21	0.90	1.590	0.670	1.241	**0.991**	6916.9
384	TGCATCACCCACATCCAGTA	528	70.68	0.70	1.513	0.496	1.127	**1.030**	5861.4
385	GCATCACCCACATCCAGTAC	529	71.39	0.70	1.617	0.496	1.191	**1.043**	8078.4
386	CATCACCCACATCCAGTACT	530	69.16	0.70	1.290	0.496	0.988	**1.013**	4148.8
387	ATCACCCACATCCAGTACTG	531	67.91	0.70	1.107	0.496	0.875	**0.913**	3317.1
388	TCACCCACATCCAGTACTGT	532	71.15	0.10	1.582	-0.025	0.971	~~0.830~~	2486.4
389	CACCCACATCCAGTACTGTT	533	69.94	-0.40	1.404	-0.460	0.696	~~0.714~~	2746.4
390	ACCCACATCCAGTACTGTTA	534	68.25	-0.40	1.157	-0.460	0.543	~~0.506~~	2133.0
391	CCCACATCCAGTACTGTTAC	535	68.25	-0.40	1.157	-0.460	0.543	~~0.297~~	2197.0
392	CCACATCCAGTACTGTTACT	536	66.50	-0.40	0.900	-0.460	0.383	~~0.066~~	1824.0
393	CACATCCAGTACTGTTACTG	537	62.61	-1.90	0.329	-1.764	-0.467	~~-0.137~~	1675.2
394	ACATCCAGTACTGTTACTGA	538	62.71	-2.30	0.344	-2.112	-0.590	~~-0.313~~	1219.8
395	CATCCAGTACTGTTACTGAT	539	62.12	-2.30	0.258	-2.112	-0.643	~~-0.504~~	1414.0
396	ATCCAGTACTGTTACTGATT	540	61.21	-2.30	0.124	-2.112	-0.726	~~-0.700~~	1710.7
397	TCCAGTACTGTTACTGATTT	541	61.58	-2.30	0.178	-2.112	-0.692	~~-0.713~~	2280.7
398	CCAGTACTGTTACTGATTTT	542	60.48	-2.30	0.017	-2.112	-0.792	~~-0.659~~	2847.7
399	CAGTACTGTTACTGATTTTT	543	56.84	-1.90	-0.518	-1.764	-0.992	~~-0.635~~	2830.2
400	AGTACTGTTACTGATTTTTT	544	55.82	-0.30	-0.666	-0.373	-0.555	~~-0.588~~	4336.3
401	GTACTGTTACTGATTTTTTC	545	57.04	0.40	-0.488	0.236	-0.213	~~-0.548~~	6581.1
402	TACTGTTACTGATTTTTTCT	546	55.95	-0.10	-0.649	-0.199	-0.478	~~-0.516~~	5406.6
403	ACTGTTACTGATTTTTTCTT	547	56.89	-0.10	-0.510	-0.199	-0.392	~~-0.450~~	6083.1
404	CTGTTACTGATTTTTTCTTT	548	56.67	-0.10	-0.542	-0.199	-0.412	~~-0.482~~	6585.7

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
405	TGTTACTGATTTTTTCTTTT	549	54.96	-0.10	-0.793	-0.199	-0.567	~~-0.575~~	3923.2
406	GTTACTGATTTTTTCTTTTT	550	55.36	-0.10	-0.734	-0.199	-0.531	~~-0.646~~	4093.5
407	TTACTGATTTTTTCTTTTTT	551	52.62	-0.10	-1.136	-0.199	-0.780	~~-0.730~~	1381.5
408	TACTGATTTTTTCTTTTTTA	552	51.70	-0.10	-1.272	-0.199	-0.864	*-0.784*	1194.3
409	ACTGATTTTTTCTTTTTTAA	553	50.45	-0.10	-1.454	-0.199	-0.977	*-0.746*	2371.3
410	CTGATTTTTTCTTTTTTAAC	554	50.45	-0.10	-1.454	-0.199	-0.977	~~-0.682~~	395.9
411	TGATTTTTTCTTTTTTAACC	555	52.50	-0.10	-1.155	-0.199	-0.792	~~-0.583~~	230.7
412	GATTTTTTCTTTTTTAACCC	556	56.43	0.30	-0.578	0.149	-0.302	~~-0.423~~	314.9
413	ATTTTTTCTTTTTTAACCCT	557	57.05	0.80	-0.487	0.583	-0.080	~~-0.246~~	276.1
414	TTTTTTCTTTTTTAACCCTG	558	56.99	0.80	-0.495	0.583	-0.085	~~-0.046~~	273.3
415	TTTTTCTTTTTTAACCCTGC	559	60.68	0.80	0.045	0.583	0.250	~~0.093~~	628.4
416	TTTTCTTTTTTAACCCTGCG	560	60.85	0.80	0.071	0.583	0.265	~~0.155~~	4661.4
417	TTTCTTTTTTAACCCTGCGG	561	62.93	0.70	0.377	0.496	0.422	~~0.167~~	411.2
418	TTCTTTTTTAACCCTGCGGG	562	65.01	-0.60	0.681	-0.634	0.181	~~0.156~~	289.5
419	TCTTTTTTAACCCTGCGGGA	563	65.91	-1.00	0.813	-0.982	0.131	~~0.130~~	244.8
420	CTTTTTTAACCCTGCGGGAT	564	64.52	-1.00	0.610	-0.982	0.005	~~0.096~~	250.7
421	TTTTTTAACCCTGCGGGATG	565	62.66	-1.00	0.337	-0.982	-0.164	~~0.067~~	207.8
422	TTTTTAACCCTGCGGGATGT	566	65.23	-1.00	0.713	-0.982	0.069	~~0.106~~	255.8
423	TTTTAACCCTGCGGGATGTG	567	64.80	-1.00	0.651	-0.982	0.030	~~0.142~~	356.8
424	TTTAACCCTGCGGGATGTGG	568	66.83	-1.00	0.949	-0.982	0.215	~~0.201~~	497.8
425	TTAACCCTGCGGGATGTGGT	569	69.50	-1.00	1.339	-0.982	0.457	~~0.318~~	754.3
426	TAACCCTGCGGGATGTGGTA	570	68.63	-1.00	1.212	-0.982	0.378	~~0.434~~	902.4
427	AACCCTGCGGGATGTGGTAT	571	69.14	-1.00	1.286	-0.982	0.424	~~0.555~~	1186.6
428	ACCCTGCGGGATGTGGTATT	572	71.66	-1.00	1.657	-0.982	0.654	~~0.595~~	1514.9
429	CCCTGCGGGATGTGGTATTC	573	72.66	-0.60	1.804	-0.634	0.878	~~0.569~~	2407.6
430	CCTGCGGGATGTGGTATTCC	574	72.66	-0.60	1.804	-0.634	0.878	~~0.526~~	3019.4
431	CTGCGGGATGTGGTATTCCT	575	71.02	-1.30	1.563	-1.243	0.497	~~0.426~~	3275.3

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
432	TGCGGGATGTGGTATTCCTA	576	68.54	-1.30	1.199	-1.243	0.271	~~0.291~~	2830.8
433	GCGGGATGTGGTATTCCTAA	577	66.48	-1.30	0.896	-1.243	0.083	~~0.108~~	2620.5
434	CGGGATGTGGTATTCCTAAT	578	62.46	-1.30	0.307	-1.243	-0.282	~~-0.058~~	1827.8
435	GGGATGTGGTATTCCTAATT	579	62.37	-1.30	0.294	-1.243	-0.290	~~-0.211~~	1957.4
436	GGATGTGGTATTCCTAATTG	580	59.71	-0.90	-0.097	-0.895	-0.400	~~-0.330~~	1686.2
437	GATGTGGTATTCCTAATTGA	581	58.45	-0.20	-0.281	-0.286	-0.283	~~-0.396~~	1395.0
438	ATGTGGTATTCCTAATTGAA	582	55.24	-0.20	-0.752	-0.286	-0.575	~~-0.444~~	1245.7
439	TGTGGTATTCCTAATTGAAC	583	55.76	-0.30	-0.675	-0.373	-0.561	~~-0.473~~	1314.0
440	GTGGTATTCCTAATTGAACT	584	57.73	-0.30	-0.387	-0.373	-0.382	~~-0.470~~	1818.7
441	TGGTATTCCTAATTGAACTT	585	55.15	-0.30	-0.765	-0.373	-0.616	~~-0.474~~	880.3
442	GGTATTCCTAATTGAACTTC	586	56.47	-0.30	-0.572	-0.373	-0.496	~~-0.413~~	1419.0
443	GTATTCCTAATTGAACTTCC	587	57.76	-0.30	-0.383	-0.373	-0.379	~~-0.343~~	1567.9
444	TATTCCTAATTGAACTTCCC	588	58.57	-0.30	-0.264	-0.373	-0.306	~~-0.248~~	1959.4
445	ATTCCTAATTGAACTTCCCA	589	60.26	-0.30	-0.016	-0.373	-0.152	~~-0.161~~	2971.8
446	TTCCTAATTGAACTTCCCAG	590	60.45	-0.10	0.013	-0.199	-0.068	~~-0.200~~	1898.5
447	TCCTAATTGAACTTCCCAGA	591	61.36	0.70	0.146	0.496	0.279	~~-0.300~~	1392.3
448	CCTAATTGAACTTCCCAGAA	592	58.27	0.70	-0.308	0.496	-0.002	~~-0.397~~	1143.2
449	CTAATTGAACTTCCCAGAAG	593	54.92	-0.70	-0.800	-0.721	-0.770	~~-0.467~~	427.7
450	TAATTGAACTTCCCAGAAGT	594	55.84	-1.90	-0.664	-1.764	-1.082	~~-0.545~~	148.5
451	AATTGAACTTCCCAGAAGTC	595	57.61	-2.10	-0.404	-1.938	-0.987	~~-0.677~~	259.1
452	ATTGAACTTCCCAGAAGTCT	596	61.42	-2.10	0.154	-1.938	-0.641	*-0.751*	241.9
453	TTGAACTTCCCAGAAGTCTT	597	61.76	-2.10	0.205	-1.938	-0.609	*-0.730*	808.1
454	TGAACTTCCCAGAAGTCTTG	598	61.34	-2.10	0.143	-1.938	-0.648	~~-0.586~~	351.6
455	GAACTTCCCAGAAGTCTTGA	599	62.71	-2.10	0.344	-1.938	-0.523	~~-0.415~~	499.7
456	AACTTCCCAGAAGTCTTGAG	600	61.63	-2.10	0.186	-1.938	-0.621	~~-0.262~~	407.4
457	ACTTCCCAGAAGTCTTGAGT	601	66.97	-1.90	0.969	-1.764	-0.069	~~-0.138~~	492.1
458	CTTCCCAGAAGTCTTGAGTT	602	66.75	-1.00	0.937	-0.982	0.208	~~-0.019~~	736.1

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
459	TTCCCAGAAGTCTTGAGTTC	603	66.31	-0.20	0.872	-0.286	0.432	~~0.058~~	815.2
460	TCCCAGAAGTCTTGAGTTCT	604	67.98	-1.20	1.116	-1.156	0.253	~~0.101~~	888.8
461	CCCAGAAGTCTTGAGTTCTC	605	67.98	-1.40	1.116	-1.330	0.187	~~0.049~~	2021.6
462	CCAGAAGTCTTGAGTTCTCT	606	66.10	-1.40	0.842	-1.330	0.017	~~-0.013~~	1988.5
463	CAGAAGTCTTGAGTTCTCTT	607	62.41	-1.40	0.300	-1.330	-0.319	~~-0.082~~	2008.8
464	AGAAGTCTTGAGTTCTCTTA	608	60.43	-1.20	0.009	-1.156	-0.434	~~-0.105~~	2631.8
465	GAAGTCTTGAGTTCTCTTAT	609	60.20	-0.50	-0.025	-0.547	-0.223	~~-0.151~~	3052.8
466	AAGTCTTGAGTTCTCTTATT	610	59.12	0.30	-0.183	0.149	-0.057	~~-0.212~~	3509.3
467	AGTCTTGAGTTCTCTTATTA	611	60.75	0.30	0.056	0.149	0.091	~~-0.211~~	3221.6
468	GTCTTGAGTTCTCTTATTAA	612	58.29	0.30	-0.305	0.149	-0.132	~~-0.216~~	3677.1
469	TCTTGAGTTCTCTTATTAAG	613	55.25	0.30	-0.751	0.149	-0.409	~~-0.238~~	1176.6
470	CTTGAGTTCTCTTATTAAGT	614	57.04	0.10	-0.488	-0.025	-0.312	~~-0.255~~	1168.1
471	TTGAGTTCTCTTATTAAGTT	615	55.29	0.10	-0.745	-0.025	-0.471	~~-0.292~~	666.3
472	TGAGTTCTCTTATTAAGTTC	616	56.35	0.10	-0.589	-0.025	-0.375	~~-0.271~~	674.0
473	GAGTTCTCTTATTAAGTTCT	617	58.57	0.10	-0.263	-0.025	-0.173	~~-0.256~~	1471.4
474	AGTTCTCTTATTAAGTTCTC	618	58.61	0.10	-0.257	-0.025	-0.169	~~-0.240~~	1493.5
475	GTTCTCTTATTAAGTTCTCT	619	60.59	0.10	0.032	-0.025	0.011	~~-0.247~~	2191.5
476	TTCTCTTATTAAGTTCTCTG	620	57.16	0.10	-0.471	-0.025	-0.301	~~-0.317~~	1410.3
477	TCTCTTATTAAGTTCTCTGA	621	58.23	0.10	-0.314	-0.025	-0.204	~~-0.413~~	1262.8
478	CTCTTATTAAGTTCTCTGAA	622	54.79	0.10	-0.817	-0.025	-0.516	~~-0.519~~	1072.9
479	TCTTATTAAGTTCTCTGAAA	623	50.95	0.10	-1.382	-0.025	-0.866	~~-0.629~~	540.9
480	CTTATTAAGTTCTCTGAAAT	624	49.77	0.50	-1.554	0.323	-0.841	~~-0.695~~	539.2
481	TTATTAAGTTCTCTGAAATC	625	48.99	0.50	-1.668	0.323	-0.912	*-0.768*	709.0
482	TATTAAGTTCTCTGAAATCT	626	50.64	0.50	-1.427	0.323	-0.762	*-0.775*	978.1
483	ATTAAGTTCTCTGAAATCTA	627	50.64	0.50	-1.427	0.323	-0.762	*-0.732*	1217.7
484	TTAAGTTCTCTGAAATCTAC	628	51.15	0.50	-1.352	0.323	-0.716	~~-0.693~~	1748.1
485	TAAGTTCTCTGAAATCTACT	629	52.79	0.50	-1.112	0.323	-0.567	~~-0.646~~	2511.5

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
486	AAGTTCTCTGAAATCTACTA	630	52.79	0.50	-1.112	0.323	-0.567	~~-0.643~~	2997.2
487	AGTTCTCTGAAATCTACTAA	631	52.79	0.50	-1.112	0.323	-0.567	~~-0.663~~	2887.6
488	GTTCTCTGAAATCTACTAAT	632	52.65	0.50	-1.133	0.323	-0.580	~~-0.725~~	4421.3
489	TTCTCTGAAATCTACTAATT	633	50.14	0.70	-1.500	0.496	-0.741	*-0.832*	1937.7
490	TCTCTGAAATCTACTAATTT	634	50.14	0.20	-1.500	0.062	-0.906	*-0.962*	1773.3
491	CTCTGAAATCTACTAATTTT	635	49.31	-0.30	-1.622	-0.373	-1.147	*-1.102*	1491.1
492	TCTGAAATCTACTAATTTTC	636	48.55	-0.60	-1.734	-0.634	-1.316	*-1.171*	376.6
493	CTGAAATCTACTAATTTTCT	637	49.31	-1.30	-1.622	-1.243	-1.478	*-1.178*	371.9
494	TGAAATCTACTAATTTTCTC	638	48.55	-1.30	-1.734	-1.243	-1.547	*-1.092*	415.2
495	GAAATCTACTAATTTTCTCC	639	52.45	-0.90	-1.161	-0.895	-1.060	*-0.938*	1097.9
496	AAATCTACTAATTTTCTCCA	640	52.47	-0.10	-1.158	-0.199	-0.794	*-0.778*	1429.1
497	AATCTACTAATTTTCTCCAT	641	54.25	0.90	-0.897	0.670	-0.301	~~-0.620~~	1812.5
498	ATCTACTAATTTTCTCCATT	642	56.46	1.00	-0.572	0.757	-0.067	~~-0.485~~	1943.4
499	TCTACTAATTTTCTCCATTT	643	56.80	0.50	-0.523	0.323	-0.202	~~-0.421~~	1506.1
500	CTACTAATTTTCTCCATTTA	644	54.93	0.50	-0.797	0.323	-0.372	~~-0.376~~	1694.7
501	TACTAATTTTCTCCATTTAG	645	53.14	0.30	-1.060	0.149	-0.600	~~-0.396~~	946.7
502	ACTAATTTTCTCCATTTAGT	646	56.69	-0.70	-0.539	-0.721	-0.608	~~-0.407~~	1114.3
503	CTAATTTTCTCCATTTAGTA	647	55.57	0.00	-0.704	-0.112	-0.479	~~-0.369~~	963.9
504	TAATTTTCTCCATTTAGTAC	648	54.12	0.50	-0.917	0.323	-0.446	~~-0.274~~	1347.9
505	AATTTTCTCCATTTAGTACT	649	56.69	0.70	-0.539	0.496	-0.145	~~-0.130~~	2067.7
506	ATTTTCTCCATTTAGTACTG	650	58.66	0.80	-0.250	0.583	0.067	~~0.037~~	2724.2
507	TTTTCTCCATTTAGTACTGT	651	61.92	0.60	0.228	0.410	0.297	~~0.186~~	3367.9
508	TTTCTCCATTTAGTACTGTC	652	63.10	0.60	0.401	0.410	0.404	~~0.314~~	5235.8
509	TTCTCCATTTAGTACTGTCT	653	64.84	0.60	0.656	0.410	0.562	~~0.377~~	6423.5
510	TCTCCATTTAGTACTGTCTT	654	64.84	0.60	0.656	0.410	0.562	~~0.396~~	7758.9
511	CTCCATTTAGTACTGTCTTT	655	63.63	0.60	0.479	0.410	0.453	~~0.342~~	8001.5
512	TCCATTTAGTACTGTCTTTT	656	61.92	0.60	0.228	0.410	0.297	~~0.273~~	5512.4

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
513	CCATTTAGTACTGTCTTTTT	657	60.78	0.60	0.061	0.410	0.194	~~0.210~~	5300.0
514	CATTTAGTACTGTCTTTTTT	658	57.04	0.80	-0.489	0.583	-0.081	~~0.147~~	3902.1
515	ATTTAGTACTGTCTTTTTTC	659	57.08	0.80	-0.482	0.583	-0.077	~~0.099~~	4641.8
516	TTTAGTACTGTCTTTTTTCT	660	59.26	0.80	-0.162	0.583	0.121	~~0.084~~	4888.4
517	TTAGTACTGTCTTTTTTCTT	661	59.26	0.80	-0.162	0.583	0.121	~~0.160~~	5477.3
518	TAGTACTGTCTTTTTTCTTT	662	59.26	0.80	-0.162	0.583	0.121	~~0.242~~	5064.9
519	AGTACTGTCTTTTTTCTTTA	663	59.26	1.00	-0.162	0.757	0.187	~~0.310~~	5580.3
520	GTACTGTCTTTTTTCTTTAT	664	59.04	2.70	-0.195	2.236	0.729	~~0.400~~	5478.3
521	TACTGTCTTTTTTCTTTATG	665	55.71	2.90	-0.683	2.410	0.492	~~0.480~~	2275.5
522	ACTGTCTTTTTTCTTTATGG	666	59.07	1.70	-0.190	1.366	0.402	~~0.524~~	1730.8
523	CTGTCTTTTTTCTTTATGGC	667	62.92	1.70	0.374	1.366	0.751	~~0.449~~	2405.5
524	TGTCTTTTTTCTTTATGGCA	668	62.14	1.70	0.260	1.366	0.680	~~0.258~~	1942.0
525	GTCTTTTTTCTTTATGGCAA	669	60.05	1.50	-0.047	1.192	0.424	~~0.068~~	2085.6
526	TCTTTTTTCTTTATGGCAAA	670	54.99	0.60	-0.788	0.410	-0.333	~~-0.106~~	493.2
527	CTTTTTTCTTTATGGCAAAT	671	53.75	0.10	-0.971	-0.025	-0.612	~~-0.309~~	532.7
528	TTTTTTCTTTATGGCAAATA	672	51.30	0.10	-1.331	-0.025	-0.835	~~-0.507~~	280.0
529	TTTTTCTTTATGGCAAATAC	673	51.49	0.10	-1.302	-0.025	-0.817	~~-0.640~~	440.8
530	TTTTCTTTATGGCAAATACT	674	53.08	0.10	-1.069	-0.025	-0.672	~~-0.652~~	463.1
531	TTTCTTTATGGCAAATACTG	675	52.74	0.10	-1.119	-0.025	-0.704	~~-0.639~~	579.0
532	TTCTTTATGGCAAATACTGG	676	54.90	0.10	-0.802	-0.025	-0.507	~~-0.572~~	673.7
533	TCTTTATGGCAAATACTGGA	677	55.85	0.10	-0.663	-0.025	-0.421	~~-0.504~~	837.0
534	CTTTATGGCAAATACTGGAG	678	54.78	0.10	-0.820	-0.025	-0.518	~~-0.490~~	1061.9
535	TTTATGGCAAATACTGGAGT	679	55.74	0.30	-0.679	0.149	-0.365	~~-0.507~~	855.0
536	TTATGGCAAATACTGGAGTA	680	54.87	0.60	-0.806	0.410	-0.344	~~-0.562~~	775.0
537	TATGGCAAATACTGGAGTAT	681	54.56	0.00	-0.852	-0.112	-0.571	~~-0.591~~	773.6
538	ATGGCAAATACTGGAGTATT	682	55.42	-1.00	-0.726	-0.982	-0.823	~~-0.647~~	702.5
539	TGGCAAATACTGGAGTATTG	683	55.37	-1.20	-0.733	-1.156	-0.893	*-0.775*	387.5

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
540	GGCAAATACTGGAGTATTGT	684	58.33	-1.20	-0.298	-1.156	-0.624	*-0.924*	435.3
541	GCAAATACTGGAGTATTGTA	685	55.24	-1.20	-0.753	-1.156	-0.906	*-0.974*	93.7
542	CAAATACTGGAGTATTGTAT	686	51.30	-1.20	-1.331	-1.156	-1.264	*-0.913*	50.0
543	AAATACTGGAGTATTGTATG	687	49.96	-1.20	-1.527	-1.156	-1.386	*-0.809*	50.4
544	AATACTGGAGTATTGTATGG	688	54.30	-1.00	-0.890	-0.982	-0.925	~~-0.688~~	64.7
545	ATACTGGAGTATTGTATGGA	689	57.60	-0.30	-0.406	-0.373	-0.394	~~-0.483~~	76.0
546	TACTGGAGTATTGTATGGAT	690	57.60	0.40	-0.406	0.236	-0.162	~~-0.236~~	86.0
547	ACTGGAGTATTGTATGGATT	691	58.53	1.30	-0.269	1.018	0.220	~~-0.009~~	123.4
548	CTGGAGTATTGTATGGATTC	692	59.39	2.00	-0.144	1.627	0.529	~~0.135~~	121.5
549	TGGAGTATTGTATGGATTCT	693	59.39	1.80	-0.144	1.453	0.463	~~0.210~~	641.3
550	GGAGTATTGTATGGATTCTC	694	60.95	0.60	0.086	0.410	0.209	~~0.286~~	161.5
551	GAGTATTGTATGGATTCTCA	695	59.52	0.60	-0.124	0.410	0.079	~~0.321~~	129.9
552	AGTATTGTATGGATTCTCAG	696	58.31	1.10	-0.302	0.844	0.134	~~0.371~~	88.7
553	GTATTGTATGGATTCTCAGG	697	60.87	1.10	0.074	0.844	0.367	~~0.462~~	112.5
554	TATTGTATGGATTCTCAGGC	698	61.97	1.10	0.236	0.844	0.467	~~0.575~~	134.6
555	ATTGTATGGATTCTCAGGCC	699	66.52	1.10	0.902	0.844	0.880	~~0.669~~	191.6
556	TTGTATGGATTCTCAGGCCC	700	70.34	0.70	1.463	0.496	1.096	~~0.714~~	254.5
557	TGTATGGATTCTCAGGCCCA	701	71.11	0.20	1.577	0.062	1.001	~~0.738~~	332.2
558	GTATGGATTCTCAGGCCCAA	702	68.95	0.00	1.259	-0.112	0.738	~~0.761~~	415.6
559	TATGGATTCTCAGGCCCAAT	703	65.78	0.00	0.795	-0.112	0.450	~~0.774~~	285.0
560	ATGGATTCTCAGGCCCAATT	704	66.68	0.00	0.925	-0.112	0.531	~~0.737~~	464.0
561	TGGATTCTCAGGCCCAATTT	705	67.04	0.20	0.979	0.062	0.630	~~0.663~~	492.5
562	GGATTCTCAGGCCCAATTTT	706	67.51	1.10	1.048	0.844	0.970	~~0.624~~	639.7
563	GATTCTCAGGCCCAATTTTT	707	65.34	1.30	0.729	1.018	0.839	~~0.595~~	512.4
564	ATTCTCAGGCCCAATTTTTG	708	63.94	0.60	0.524	0.410	0.481	~~0.513~~	393.4
565	TTCTCAGGCCCAATTTTTGA	709	65.24	0.20	0.716	0.062	0.467	~~0.394~~	334.3
566	TCTCAGGCCCAATTTTTGAA	710	62.85	0.20	0.364	0.062	0.249	~~0.181~~	308.2

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
567	CTCAGGCCCAATTTTTGAAA	711	59.62	0.20	-0.109	0.062	-0.044	~~-0.048~~	199.2
568	TCAGGCCCAATTTTTGAAAT	712	57.85	0.20	-0.369	0.062	-0.205	~~-0.223~~	164.3
569	CAGGCCCAATTTTTGAAATT	713	56.95	-0.50	-0.501	-0.547	-0.518	~~-0.412~~	125.6
570	AGGCCCAATTTTTGAAATTT	714	56.09	-1.00	-0.627	-0.982	-0.762	~~-0.571~~	102.6
571	GGCCCAATTTTTGAAATTTT	715	56.23	-1.00	-0.606	-0.982	-0.749	~~-0.688~~	91.6
572	GCCCAATTTTTGAAATTTTC	716	55.07	-1.00	-0.777	-0.982	-0.855	*-0.806*	76.2
573	CCCAATTTTTGAAATTTTCC	717	54.96	-1.00	-0.792	-0.982	-0.864	*-0.881*	78.8
574	CCAATTTTTGAAATTTTCCC	718	54.96	-1.00	-0.792	-0.982	-0.864	*-0.841*	84.8
575	CAATTTTTGAAATTTTCCCT	719	53.17	-1.00	-1.055	-0.982	-1.027	*-0.755*	162.0
576	AATTTTTGAAATTTTCCCTT	720	52.25	-0.80	-1.190	-0.808	-1.045	~~-0.634~~	539.5
577	ATTTTTGAAATTTTCCCTTC	721	55.17	0.10	-0.762	-0.025	-0.482	~~-0.511~~	1787.3
578	TTTTTGAAATTTTCCCTTCC	722	58.88	0.10	-0.219	-0.025	-0.145	~~-0.389~~	6354.2
579	TTTTGAAATTTTCCCTTCCT	723	60.39	0.10	0.004	-0.025	-0.007	~~-0.243~~	9513.6
580	TTTGAAATTTTCCCTTCCTT	724	60.39	0.10	0.004	-0.025	-0.007	~~-0.062~~	10660.0
581	TTGAAATTTTCCCTTCCTTT	725	60.39	0.10	0.004	-0.025	-0.007	~~0.107~~	11202.0
582	TGAAATTTTCCCTTCCTTTT	726	60.39	0.10	0.004	-0.025	-0.007	~~0.293~~	11543.0
583	GAAATTTTCCCTTCCTTTTC	727	61.81	0.40	0.212	0.236	0.221	~~0.596~~	14774.0
584	AAATTTTCCCTTCCTTTTCC	728	64.17	1.20	0.557	0.931	0.699	**0.952**	18197.0
585	AATTTTCCCTTCCTTTTCCA	729	67.39	1.70	1.030	1.366	1.158	**1.307**	21410.0
586	ATTTTCCCTTCCTTTTCCAT	730	69.58	4.00	1.351	3.366	2.117	**1.679**	22869.0
587	TTTTCCCTTCCTTTTCCATT	731	69.96	5.00	1.408	4.236	2.482	**2.039**	21818.0
588	TTTCCCTTCCTTTTCCATTT	732	69.96	5.00	1.408	4.236	2.482	**2.113**	21341.0
589	TTCCCTTCCTTTTCCATTTC	733	71.19	5.00	1.588	4.236	2.594	**2.085**	22063.0
590	TCCCTTCCTTTTCCATTTCT	734	72.77	5.00	1.820	4.236	2.738	**1.863**	22152.0
591	CCCTTCCTTTTCCATTTCTG	735	71.01	0.90	1.561	0.670	1.223	**1.571**	20764.0
592	CCTTCCTTTTCCATTTCTGT	736	70.68	0.20	1.513	0.062	0.961	**1.289**	12579.0
593	CTTCCTTTTCCATTTCTGTA	737	66.30	0.20	0.870	0.062	0.563	**0.945**	9036.3

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
594	TTCCTTTTCCATTTCTGTAC	738	64.87	0.20	0.660	0.062	0.433	~~0.505~~	8251.8
595	TCCTTTTCCATTTCTGTACA	739	65.74	0.20	0.788	0.062	0.512	~~0.257~~	20788.0
596	CCTTTTCCATTTCTGTACAA	740	62.11	0.20	0.256	0.062	0.182	~~0.024~~	7073.9
597	CTTTTCCATTTCTGTACAAA	741	56.39	0.20	-0.583	0.062	-0.338	~~-0.153~~	2932.4
598	TTTTCCATTTCTGTACAAAT	742	54.49	0.20	-0.862	0.062	-0.511	~~-0.300~~	1897.3
599	TTTCCATTTCTGTACAAATT	743	54.49	-0.30	-0.862	-0.373	-0.676	~~-0.449~~	2158.1
600	TTCCATTTCTGTACAAATTT	744	54.49	-0.30	-0.862	-0.373	-0.676	~~-0.608~~	2215.9
601	TCCATTTCTGTACAAATTTC	745	55.43	-0.30	-0.724	-0.373	-0.591	~~-0.695~~	2168.6
602	CCATTTCTGTACAAATTTCT	746	56.07	-0.30	-0.631	-0.373	-0.533	~~-0.708~~	2025.8
603	CATTTCTGTACAAATTTCTA	747	51.65	-0.30	-1.278	-0.373	-0.934	~~-0.708~~	1277.2
604	ATTTCTGTACAAATTTCTAC	748	50.83	-0.10	-1.398	-0.199	-0.943	*-0.736*	1944.8
605	TTTCTGTACAAATTTCTACT	749	52.78	0.40	-1.112	0.236	-0.600	*-0.790*	2504.3
606	TTCTGTACAAATTTCTACTA	750	51.90	0.40	-1.242	0.236	-0.681	*-0.876*	2941.5
607	TCTGTACAAATTTCTACTAA	751	49.84	0.40	-1.544	0.236	-0.868	*-0.846*	2694.8
608	CTGTACAAATTTCTACTAAT	752	48.73	0.40	-1.707	0.236	-0.969	*-0.827*	2610.7
609	TGTACAAATTTCTACTAATG	753	46.88	0.40	-1.979	0.236	-1.137	*-0.845*	1678.1
610	GTACAAATTTCTACTAATGC	754	50.66	0.60	-1.424	0.410	-0.727	*-0.854*	5877.3
611	TACAAATTTCTACTAATGCT	755	49.82	0.60	-1.547	0.410	-0.803	*-0.849*	4461.0
612	ACAAATTTCTACTAATGCTT	756	50.65	0.60	-1.425	0.410	-0.728	*-0.816*	5943.2
613	CAAATTTCTACTAATGCTTT	757	50.46	0.60	-1.453	0.410	-0.745	*-0.753*	6492.9
614	AAATTTCTACTAATGCTTTT	758	49.47	0.60	-1.599	0.410	-0.836	*-0.745*	6875.0
615	AATTTCTACTAATGCTTTTA	759	50.61	0.60	-1.431	0.410	-0.731	~~-0.727~~	7950.3
616	ATTTCTACTAATGCTTTTAT	760	52.40	0.20	-1.169	0.062	-0.701	~~-0.719~~	8314.8
617	TTTCTACTAATGCTTTTATT	761	52.72	0.20	-1.122	0.062	-0.672	~~-0.720~~	6885.8
618	TTCTACTAATGCTTTTATTT	762	52.72	0.20	-1.122	0.062	-0.672	~~-0.730~~	6443.2
619	TCTACTAATGCTTTTATTTT	763	52.72	0.20	-1.122	0.062	-0.672	*-0.731*	6331.0
620	CTACTAATGCTTTTATTTTT	764	51.81	0.20	-1.255	0.062	-0.755	~~-0.718~~	5952.5

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
621	TACTAATGCTTTTATTTTTT	765	50.18	0.20	-1.494	0.062	-0.903	~~-0.721~~	2662.8
622	ACTAATGCTTTTATTTTTTC	766	51.96	0.20	-1.233	0.062	-0.741	~~-0.667~~	3034.0
623	CTAATGCTTTTATTTTTTCT	767	53.41	0.20	-1.021	0.062	-0.609	~~-0.513~~	2198.5
624	TAATGCTTTTATTTTTTCTT	768	51.76	0.40	-1.263	0.236	-0.694	~~-0.315~~	1670.1
625	AATGCTTTTATTTTTTCTTC	769	53.61	1.10	-0.992	0.844	-0.294	~~-0.038~~	3039.4
626	ATGCTTTTATTTTTTCTTCT	770	57.66	2.10	-0.397	1.714	0.405	~~0.177~~	3873.8
627	TGCTTTTATTTTTTCTTCTG	771	57.60	2.80	-0.406	2.323	0.631	~~0.363~~	3609.7
628	GCTTTTATTTTTTCTTCTGT	772	60.96	3.10	0.087	2.583	1.036	~~0.464~~	4891.4
629	CTTTTATTTTTTCTTCTGTC	773	57.96	3.10	-0.353	2.583	0.763	~~0.480~~	3071.6
630	TTTTATTTTTTCTTCTGTCA	774	57.22	3.10	-0.461	2.583	0.696	~~0.391~~	2667.2
631	TTTATTTTTTCTTCTGTCAA	775	54.81	1.70	-0.816	1.366	0.013	~~0.312~~	2293.1
632	TTATTTTTTCTTCTGTCAAT	776	54.46	1.20	-0.866	0.931	-0.183	~~0.232~~	2123.0
633	TATTTTTTCTTCTGTCAATG	777	54.08	1.20	-0.922	0.931	-0.218	~~0.237~~	1914.7
634	ATTTTTTCTTCTGTCAATGG	778	57.36	1.20	-0.442	0.931	0.080	~~0.263~~	2174.1
635	TTTTTTCTTCTGTCAATGGC	779	61.67	1.20	0.192	0.931	0.473	~~0.372~~	3659.7
636	TTTTTCTTCTGTCAATGGCC	780	65.26	1.20	0.717	0.931	0.799	~~0.509~~	5217.7
637	TTTTCTTCTGTCAATGGCCA	781	66.11	1.20	0.843	0.931	0.877	~~0.569~~	4559.7
638	TTTCTTCTGTCAATGGCCAT	782	65.73	1.00	0.787	0.757	0.776	~~0.576~~	4347.7
639	TTCTTCTGTCAATGGCCATT	783	65.73	1.00	0.787	0.757	0.776	~~0.506~~	5267.4
640	TCTTCTGTCAATGGCCATTG	784	65.26	-0.60	0.718	-0.634	0.204	~~0.389~~	3922.8
641	CTTCTGTCAATGGCCATTGT	785	66.97	-1.30	0.968	-1.243	0.128	~~0.235~~	3608.6
642	TTCTGTCAATGGCCATTGTT	786	65.36	-1.30	0.733	-1.243	-0.018	~~0.044~~	1881.6
643	TCTGTCAATGGCCATTGTTT	787	65.36	-1.30	0.733	-1.243	-0.018	~~-0.139~~	1658.0
644	CTGTCAATGGCCATTGTTTA	788	63.32	-1.30	0.433	-1.243	-0.204	~~-0.255~~	1369.8
645	TGTCAATGGCCATTGTTTAA	789	59.38	-1.30	-0.144	-1.243	-0.562	~~-0.353~~	605.8
646	GTCAATGGCCATTGTTTAAC	790	59.99	-1.30	-0.055	-1.243	-0.506	~~-0.357~~	933.2
647	TCAATGGCCATTGTTTAACT	791	58.93	-1.30	-0.211	-1.243	-0.603	~~-0.331~~	441.8

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
648	CAATGGCCATTGTTTAACTT	792	57.97	-0.90	-0.352	-0.895	-0.558	~~-0.281~~	545.6
649	AATGGCCATTGTTTAACTTT	793	57.07	0.90	-0.483	0.670	-0.045	~~-0.173~~	781.4
650	ATGGCCATTGTTTAACTTTT	794	59.31	0.90	-0.156	0.670	0.158	~~-0.092~~	1027.3
651	TGGCCATTGTTTAACTTTTG	795	59.24	0.90	-0.165	0.670	0.152	~~0.021~~	1102.5
652	GGCCATTGTTTAACTTTTGG	796	61.84	0.30	0.216	0.149	0.190	~~0.156~~	935.7
653	GCCATTGTTTAACTTTTGGG	797	61.84	-0.10	0.216	-0.199	0.058	~~0.218~~	403.7
654	CCATTGTTTAACTTTTGGGC	798	61.84	0.30	0.216	0.149	0.190	~~0.251~~	269.3
655	CATTGTTTAACTTTTGGGCC	799	61.84	0.90	0.216	0.670	0.389	~~0.299~~	296.8
656	ATTGTTTAACTTTTGGGCCA	800	61.84	0.90	0.216	0.670	0.389	~~0.367~~	449.4
657	TTGTTTAACTTTTGGGCCAT	801	61.84	0.90	0.216	0.670	0.389	~~0.377~~	448.1
658	TGTTTAACTTTTGGGCCATC	802	62.91	0.90	0.373	0.670	0.486	~~0.340~~	584.9
659	GTTTAACTTTTGGGCCATCC	803	66.73	0.40	0.934	0.236	0.669	~~0.275~~	1032.4
660	TTTAACTTTTGGGCCATCCA	804	64.79	-0.70	0.649	-0.721	0.128	~~0.235~~	737.8
661	TTAACTTTTGGGCCATCCAT	805	64.44	-1.20	0.598	-1.156	-0.069	~~0.271~~	950.2
662	TAACTTTTGGGCCATCCATT	806	64.44	-1.20	0.598	-1.156	-0.069	~~0.310~~	1308.0
663	AACTTTTGGGCCATCCATTC	807	66.42	-1.20	0.888	-1.156	0.111	~~0.296~~	2360.1
664	ACTTTTGGGCCATCCATTCC	808	72.21	-1.20	1.738	-1.156	0.638	~~0.387~~	4946.0
665	CTTTTGGGCCATCCATTCCT	809	73.53	-1.20	1.930	-1.156	0.758	~~0.480~~	6789.2
666	TTTTGGGCCATCCATTCCTG	810	71.49	-1.20	1.632	-1.156	0.573	~~0.560~~	8150.6
667	TTTGGGCCATCCATTCCTGG	811	73.62	-1.20	1.945	-1.156	0.766	~~0.622~~	7589.0
668	TTGGGCCATCCATTCCTGGC	812	77.43	-2.80	2.504	-2.547	0.584	~~0.580~~	13914.0
669	TGGGCCATCCATTCCTGGCT	813	78.94	-3.50	2.725	-3.156	0.490	~~0.500~~	17513.0
670	GGGCCATCCATTCCTGGCTT	814	79.51	-3.50	2.809	-3.156	0.542	~~0.449~~	19883.0
671	GGCCATCCATTCCTGGCTTT	815	77.37	-3.50	2.494	-3.156	0.347	~~0.324~~	20103.0
672	GCCATCCATTCCTGGCTTTA	816	74.28	-3.10	2.040	-2.808	0.198	~~0.214~~	18622.0
673	CCATCCATTCCTGGCTTTAA	817	67.92	-1.30	1.109	-1.243	0.215	~~0.122~~	16915.0
674	CATCCATTCCTGGCTTTAAT	818	64.36	-1.30	0.585	-1.243	-0.109	~~0.028~~	13910.0

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
675	ATCCATTCCTGGCTTTAATT	819	63.53	-1.30	0.464	-1.243	-0.185	~~-0.009~~	12524.0
676	TCCATTCCTGGCTTTAATTT	820	63.88	-1.30	0.516	-1.243	-0.152	~~-0.005~~	11890.0
677	CCATTCCTGGCTTTAATTTT	821	62.81	-0.90	0.359	-0.895	-0.118	~~0.040~~	12839.0
678	CATTCCTGGCTTTAATTTTA	822	58.55	0.90	-0.266	0.670	0.090	~~0.126~~	9726.8
679	ATTCCTGGCTTTAATTTTAC	823	57.84	1.50	-0.371	1.192	0.223	~~0.238~~	8499.7
680	TTCCTGGCTTTAATTTTACT	824	59.78	1.90	-0.086	1.540	0.532	~~0.336~~	6800.4
681	TCCTGGCTTTAATTTTACTG	825	59.37	1.90	-0.146	1.540	0.494	~~0.396~~	5445.6
682	CCTGGCTTTAATTTTACTGG	826	60.53	1.90	0.024	1.540	0.600	~~0.434~~	2901.6
683	CTGGCTTTAATTTTACTGGT	827	59.77	1.90	-0.087	1.540	0.531	~~0.431~~	1174.2
684	TGGCTTTAATTTTACTGGTA	828	57.25	1.90	-0.458	1.540	0.301	~~0.268~~	521.3
685	GGCTTTAATTTTACTGGTAC	829	57.86	1.90	-0.368	1.540	0.357	~~0.066~~	611.1
686	GCTTTAATTTTACTGGTACA	830	56.55	1.80	-0.560	1.453	0.205	~~-0.148~~	287.6
687	CTTTAATTTTACTGGTACAG	831	52.66	0.40	-1.130	0.236	-0.611	~~-0.330~~	109.5
688	TTTAATTTTACTGGTACAGT	832	53.62	-0.80	-0.989	-0.808	-0.920	~~-0.454~~	59.5
689	TTAATTTTACTGGTACAGTC	833	54.59	-1.00	-0.847	-0.982	-0.898	~~-0.540~~	62.1
690	TAATTTTACTGGTACAGTCT	834	56.28	-1.00	-0.599	-0.982	-0.745	~~-0.632~~	59.4
691	AATTTTACTGGTACAGTCTC	835	58.27	-1.00	-0.308	-0.982	-0.564	~~-0.613~~	68.0
692	ATTTTACTGGTACAGTCTCA	836	61.78	-1.00	0.207	-0.982	-0.245	~~-0.561~~	72.9
693	TTTTACTGGTACAGTCTCAA	837	59.61	-1.00	-0.111	-0.982	-0.442	~~-0.515~~	62.2
694	TTTACTGGTACAGTCTCAAT	838	59.25	-1.00	-0.164	-0.982	-0.475	~~-0.439~~	64.5
695	TTACTGGTACAGTCTCAATA	839	58.30	-1.00	-0.303	-0.982	-0.561	~~-0.318~~	53.5
696	TACTGGTACAGTCTCAATAG	840	58.15	-1.00	-0.326	-0.982	-0.575	~~-0.166~~	57.8
697	ACTGGTACAGTCTCAATAGG	841	61.44	-0.80	0.157	-0.808	-0.210	~~0.034~~	341.0
698	CTGGTACAGTCTCAATAGGG	842	63.55	0.10	0.467	-0.025	0.280	~~0.186~~	54.8
699	TGGTACAGTCTCAATAGGGC	843	65.89	1.10	0.810	0.844	0.823	~~0.279~~	47.1
700	GGTACAGTCTCAATAGGGCT	844	68.08	0.90	1.131	0.670	0.956	~~0.383~~	59.7
701	GTACAGTCTCAATAGGGCTA	845	64.73	0.70	0.640	0.496	0.586	~~0.425~~	47.0

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
702	TACAGTCTCAATAGGGCTAA	846	59.35	0.70	-0.149	0.496	0.096	~~0.425~~	49.3
703	ACAGTCTCAATAGGGCTAAT	847	59.91	0.70	-0.067	0.496	0.147	~~0.388~~	55.0
704	CAGTCTCAATAGGGCTAATG	848	59.29	0.70	-0.158	0.496	0.091	~~0.275~~	49.0
705	AGTCTCAATAGGGCTAATGG	849	60.62	0.90	0.037	0.670	0.278	~~0.220~~	45.7
706	GTCTCAATAGGGCTAATGGG	850	63.00	1.10	0.386	0.844	0.560	~~0.189~~	115.6
707	TCTCAATAGGGCTAATGGGA	851	61.22	0.40	0.125	0.236	0.167	~~0.133~~	50.6
708	CTCAATAGGGCTAATGGGAA	852	57.97	1.40	-0.352	1.105	0.202	~~0.075~~	48.0
709	TCAATAGGGCTAATGGGAAA	853	54.39	1.40	-0.877	1.105	-0.124	~~-0.028~~	50.5
710	CAATAGGGCTAATGGGAAAA	854	51.64	1.80	-1.281	1.453	-0.242	~~-0.191~~	44.1
711	AATAGGGCTAATGGGAAAAT	855	50.45	1.90	-1.454	1.540	-0.316	~~-0.298~~	43.1
712	ATAGGGCTAATGGGAAAATT	856	52.34	1.00	-1.178	0.757	-0.442	~~-0.432~~	45.2
713	TAGGGCTAATGGGAAAATTT	857	52.63	0.50	-1.135	0.323	-0.581	~~-0.569~~	47.4
714	AGGGCTAATGGGAAAATTTA	858	52.63	0.50	-1.135	0.323	-0.581	~~-0.717~~	50.0
715	GGGCTAATGGGAAAATTTAA	859	50.89	0.50	-1.390	0.323	-0.739	*-0.867*	47.8
716	GGCTAATGGGAAAATTTAAA	860	47.14	0.50	-1.940	0.323	-1.080	*-1.022*	50.2
717	GCTAATGGGAAAATTTAAAG	861	45.00	0.50	-2.254	0.323	-1.275	*-1.096*	43.0
718	CTAATGGGAAAATTTAAAGT	862	43.95	0.50	-2.408	0.323	-1.371	*-1.088*	57.0
719	TAATGGGAAAATTTAAAGTG	863	42.27	0.50	-2.655	0.323	-1.524	*-1.072*	58.7
720	AATGGGAAAATTTAAAGTGC	864	46.18	0.70	-2.081	0.496	-1.102	*-1.011*	183.6
721	ATGGGAAAATTTAAAGTGCA	865	48.90	1.70	-1.682	1.366	-0.524	*-0.924*	303.4
722	TGGGAAAATTTAAAGTGCAA	866	47.39	1.80	-1.903	1.453	-0.628	*-0.837*	135.7
723	GGGAAAATTTAAAGTGCAAC	867	47.84	1.60	-1.838	1.279	-0.653	*-0.766*	241.7
724	GGAAAATTTAAAGTGCAACC	868	49.12	1.20	-1.649	0.931	-0.669	*-0.737*	132.5
725	GAAAATTTAAAGTGCAACCA	869	48.09	1.20	-1.801	0.931	-0.763	*-0.758*	128.8
726	AAAATTTAAAGTGCAACCAA	870	45.57	1.10	-2.171	0.844	-1.025	~~-0.720~~	141.0
727	AAATTTAAAGTGCAACCAAT	871	46.97	1.10	-1.965	0.844	-0.897	~~-0.679~~	282.0
728	AATTTAAAGTGCAACCAATC	872	49.46	1.10	-1.599	0.844	-0.671	~~-0.629~~	948.6

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
729	ATTTAAAGTGCAACCAATCT	873	52.84	1.10	-1.104	0.844	-0.363	~~-0.567~~	1815.1
730	TTTAAAGTGCAACCAATCTG	874	52.81	1.10	-1.109	0.844	-0.366	~~-0.426~~	3188.2
731	TTAAAGTGCAACCAATCTGA	875	53.71	1.00	-0.976	0.757	-0.317	~~-0.262~~	3566.1
732	TAAAGTGCAACCAATCTGAG	876	53.56	1.00	-0.999	0.757	-0.331	~~-0.087~~	2925.1
733	AAAGTGCAACCAATCTGAGT	877	56.81	1.00	-0.522	0.757	-0.036	~~0.014~~	3233.2
734	AAGTGCAACCAATCTGAGTC	878	59.99	1.00	-0.055	0.757	0.254	~~0.085~~	3615.6
735	AGTGCAACCAATCTGAGTCA	879	63.25	1.00	0.422	0.757	0.550	~~0.165~~	3994.8
736	GTGCAACCAATCTGAGTCAA	880	61.00	1.00	0.093	0.757	0.345	~~0.138~~	4033.0
737	TGCAACCAATCTGAGTCAAC	881	58.62	1.00	-0.257	0.757	0.128	~~0.008~~	3380.2
738	GCAACCAATCTGAGTCAACA	882	59.87	1.00	-0.073	0.757	0.242	~~-0.173~~	4288.7
739	CAACCAATCTGAGTCAACAG	883	56.22	-0.30	-0.608	-0.373	-0.519	~~-0.445~~	744.1
740	AACCAATCTGAGTCAACAGA	884	56.24	-1.60	-0.605	-1.504	-0.946	*-0.757*	392.2
741	ACCAATCTGAGTCAACAGAT	885	58.10	-2.30	-0.332	-2.112	-1.009	*-1.030*	158.1
742	CCAATCTGAGTCAACAGATT	886	57.90	-3.30	-0.362	-2.982	-1.357	*-1.219*	70.8
743	CAATCTGAGTCAACAGATTT	887	54.41	-3.80	-0.874	-3.417	-1.840	*-1.262*	190.0
744	AATCTGAGTCAACAGATTTC	888	54.37	-3.60	-0.880	-3.243	-1.778	*-1.168*	87.7
745	ATCTGAGTCAACAGATTTCT	889	58.37	-2.60	-0.293	-2.373	-1.084	*-1.017*	152.7
746	TCTGAGTCAACAGATTTCTT	890	58.73	-1.90	-0.241	-1.764	-0.820	*-0.797*	270.5
747	CTGAGTCAACAGATTTCTTC	891	58.73	-0.30	-0.241	-0.373	-0.291	~~-0.553~~	498.7
748	TGAGTCAACAGATTTCTTCC	892	60.70	0.20	0.049	0.062	0.054	~~-0.321~~	891.0
749	GAGTCAACAGATTTCTTCCA	893	62.06	0.20	0.248	0.062	0.177	~~-0.221~~	1509.8
750	AGTCAACAGATTTCTTCCAA	894	58.66	0.20	-0.250	0.062	-0.132	~~-0.182~~	1009.3
751	GTCAACAGATTTCTTCCAAT	895	58.47	0.20	-0.279	0.062	-0.149	~~-0.235~~	1198.0
752	TCAACAGATTTCTTCCAATT	896	55.86	0.20	-0.661	0.062	-0.387	~~-0.315~~	680.5
753	CAACAGATTTCTTCCAATTA	897	54.08	0.20	-0.922	0.062	-0.548	~~-0.381~~	762.5
754	AACAGATTTCTTCCAATTAT	898	52.82	0.20	-1.107	0.062	-0.663	~~-0.415~~	689.8
755	ACAGATTTCTTCCAATTATG	899	54.58	0.20	-0.849	0.062	-0.503	~~-0.445~~	715.1

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
756	CAGATTTCTTCCAATTATGT	900	56.99	0.20	-0.496	0.062	-0.284	~~-0.460~~	833.8
757	AGATTTCTTCCAATTATGTT	901	56.02	0.20	-0.638	0.062	-0.372	~~-0.445~~	1067.7
758	GATTTCTTCCAATTATGTTG	902	55.80	0.30	-0.670	0.149	-0.359	~~-0.401~~	1225.9
759	ATTTCTTCCAATTATGTTGA	903	55.80	-0.10	-0.670	-0.199	-0.491	~~-0.382~~	1028.7
760	TTTCTTCCAATTATGTTGAC	904	56.34	-0.10	-0.591	-0.199	-0.442	~~-0.378~~	1419.0
761	TTCTTCCAATTATGTTGACA	905	57.29	-0.10	-0.452	-0.199	-0.356	~~-0.348~~	1437.4
762	TCTTCCAATTATGTTGACAG	906	57.14	-0.10	-0.474	-0.199	-0.369	~~-0.325~~	1518.3
763	CTTCCAATTATGTTGACAGG	907	58.36	-0.10	-0.295	-0.199	-0.259	~~-0.262~~	1560.3
764	TTCCAATTATGTTGACAGGT	908	59.43	-0.10	-0.138	-0.199	-0.161	~~-0.244~~	1100.0
765	TCCAATTATGTTGACAGGTG	909	59.02	-0.10	-0.198	-0.199	-0.198	~~-0.216~~	1096.4
766	CCAATTATGTTGACAGGTGT	910	60.68	-0.10	0.046	-0.199	-0.047	~~-0.124~~	1103.4
767	CAATTATGTTGACAGGTGTA	911	56.24	0.30	-0.605	0.149	-0.319	~~-0.005~~	738.1
768	AATTATGTTGACAGGTGTAG	912	55.09	1.10	-0.774	0.844	-0.159	~~0.054~~	596.7
769	ATTATGTTGACAGGTGTAGG	913	59.83	1.10	-0.079	0.844	0.272	~~0.161~~	548.1
770	TTATGTTGACAGGTGTAGGT	914	63.16	1.10	0.409	0.844	0.575	~~0.274~~	701.1
771	TATGTTGACAGGTGTAGGTC	915	64.38	-0.20	0.588	-0.286	0.256	~~0.420~~	724.7
772	ATGTTGACAGGTGTAGGTCC	916	69.08	-0.60	1.278	-0.634	0.551	~~0.506~~	1129.8
773	TGTTGACAGGTGTAGGTCCT	917	71.21	-0.60	1.591	-0.634	0.745	~~0.537~~	1214.0
774	GTTGACAGGTGTAGGTCCTA	918	70.75	-0.60	1.523	-0.634	0.703	~~0.520~~	1425.4
775	TTGACAGGTGTAGGTCCTAC	919	67.83	-0.60	1.095	-0.634	0.438	~~0.499~~	838.8
776	TGACAGGTGTAGGTCCTACT	920	69.52	-0.90	1.343	-0.895	0.493	~~0.427~~	1173.1
777	GACAGGTGTAGGTCCTACTA	921	69.06	-0.90	1.275	-0.895	0.450	~~0.304~~	1367.0
778	ACAGGTGTAGGTCCTACTAA	922	65.30	-0.90	0.723	-0.895	0.108	~~0.192~~	872.0
779	CAGGTGTAGGTCCTACTAAT	923	64.69	-0.90	0.634	-0.895	0.053	~~0.109~~	897.6
780	AGGTGTAGGTCCTACTAATA	924	62.84	-0.90	0.362	-0.895	-0.115	~~-0.024~~	962.2
781	GGTGTAGGTCCTACTAATAC	925	63.19	-0.90	0.414	-0.895	-0.083	~~-0.090~~	1382.6
782	GTGTAGGTCCTACTAATACT	926	62.53	-0.90	0.317	-0.895	-0.143	~~-0.099~~	1132.9

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
783	TGTAGGTCCTACTAATACTG	927	59.27	-0.90	-0.160	-0.895	-0.439	~~-0.095~~	1180.7
784	GTAGGTCCTACTAATACTGT	928	62.53	-0.50	0.317	-0.547	-0.011	~~-0.020~~	1932.9
785	TAGGTCCTACTAATACTGTA	929	58.77	0.70	-0.234	0.496	0.043	~~0.042~~	1634.4
786	AGGTCCTACTAATACTGTAC	930	59.91	0.50	-0.067	0.323	0.081	~~0.067~~	2488.1
787	GGTCCTACTAATACTGTACC	931	63.54	0.50	0.466	0.323	0.411	~~0.116~~	3560.9
788	GTCCTACTAATACTGTACCT	932	62.91	0.50	0.373	0.323	0.354	~~0.048~~	3850.1
789	TCCTACTAATACTGTACCTA	933	59.31	0.50	-0.155	0.323	0.026	~~-0.041~~	1879.0
790	CCTACTAATACTGTACCTAT	934	57.99	0.50	-0.348	0.323	-0.093	~~-0.053~~	1920.4
791	CTACTAATACTGTACCTATA	935	53.68	0.50	-0.981	0.323	-0.486	~~-0.094~~	1131.2
792	TACTAATACTGTACCTATAG	936	51.92	0.70	-1.240	0.496	-0.580	~~-0.147~~	756.5
793	ACTAATACTGTACCTATAGC	937	56.45	1.20	-0.574	0.931	-0.002	~~-0.142~~	1881.3
794	CTAATACTGTACCTATAGCT	938	57.85	1.20	-0.369	0.931	0.125	~~-0.102~~	2033.6
795	TAATACTGTACCTATAGCTT	939	56.25	1.20	-0.604	0.931	-0.021	~~-0.006~~	1853.9
796	AATACTGTACCTATAGCTTT	940	57.14	1.20	-0.473	0.931	0.060	~~0.111~~	2462.6
797	ATACTGTACCTATAGCTTTA	941	58.55	1.20	-0.266	0.931	0.189	~~0.183~~	2436.8
798	TACTGTACCTATAGCTTTAT	942	58.55	1.20	-0.266	0.931	0.189	~~0.220~~	1865.2
799	ACTGTACCTATAGCTTTATG	943	59.06	1.20	-0.192	0.931	0.235	~~0.331~~	1682.1
800	CTGTACCTATAGCTTTATGT	944	61.64	1.30	0.187	1.018	0.503	~~0.405~~	1551.3
801	TGTACCTATAGCTTTATGTC	945	61.08	1.10	0.105	0.844	0.386	~~0.484~~	1600.1
802	GTACCTATAGCTTTATGTCC	946	65.16	1.10	0.703	0.844	0.757	~~0.572~~	4094.6
803	TACCTATAGCTTTATGTCCA	947	63.16	1.10	0.409	0.844	0.575	~~0.597~~	2794.2
804	ACCTATAGCTTTATGTCCAC	948	64.30	1.30	0.577	1.018	0.745	~~0.575~~	4754.9
805	CCTATAGCTTTATGTCCACA	949	64.94	1.30	0.671	1.018	0.803	~~0.554~~	4185.4
806	CTATAGCTTTATGTCCACAG	950	61.34	1.10	0.143	0.844	0.409	~~0.484~~	3284.3
807	TATAGCTTTATGTCCACAGA	951	60.70	1.10	0.048	0.844	0.351	~~0.453~~	2819.7
808	ATAGCTTTATGTCCACAGAT	952	61.27	0.60	0.132	0.410	0.238	~~0.414~~	3545.1
809	TAGCTTTATGTCCACAGATT	953	61.63	0.60	0.186	0.410	0.271	~~0.337~~	4232.6

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
810	AGCTTTATGTCCACAGATTT	954	62.57	0.60	0.324	0.410	0.356	~~0.283~~	5252.8
811	GCTTTATGTCCACAGATTTC	955	63.85	0.60	0.511	0.410	0.472	~~0.232~~	6823.9
812	CTTTATGTCCACAGATTTCT	956	61.56	0.60	0.176	0.410	0.265	~~0.193~~	4829.8
813	TTTATGTCCACAGATTTCTA	957	58.97	0.60	-0.205	0.410	0.029	~~0.173~~	4333.7
814	TTATGTCCACAGATTTCTAT	958	58.62	0.60	-0.257	0.410	-0.004	~~0.144~~	3801.0
815	TATGTCCACAGATTTCTATG	959	58.20	0.60	-0.318	0.410	-0.041	~~0.142~~	3528.2
816	ATGTCCACAGATTTCTATGA	960	60.12	0.60	-0.036	0.410	0.134	~~0.129~~	2080.0
817	TGTCCACAGATTTCTATGAG	961	60.34	0.60	-0.004	0.410	0.153	~~0.145~~	913.8
818	GTCCACAGATTTCTATGAGT	962	63.68	0.60	0.486	0.410	0.457	~~0.122~~	1228.3
819	TCCACAGATTTCTATGAGTA	963	59.83	0.80	-0.078	0.583	0.173	~~0.067~~	238.1
820	CCACAGATTTCTATGAGTAT	964	58.43	1.10	-0.285	0.844	0.144	~~-0.078~~	219.4
821	CACAGATTTCTATGAGTATC	965	55.78	0.90	-0.673	0.670	-0.162	~~-0.169~~	138.6
822	ACAGATTTCTATGAGTATCT	966	56.48	-0.10	-0.571	-0.199	-0.430	~~-0.273~~	112.7
823	CAGATTTCTATGAGTATCTG	967	55.85	-1.30	-0.663	-1.243	-0.883	~~-0.327~~	133.8
824	AGATTTCTATGAGTATCTGA	968	55.87	-0.10	-0.659	-0.199	-0.485	~~-0.387~~	296.8
825	GATTTCTATGAGTATCTGAT	969	55.69	0.60	-0.686	0.410	-0.270	~~-0.442~~	279.7
826	ATTTCTATGAGTATCTGATC	970	55.67	0.80	-0.689	0.583	-0.206	~~-0.498~~	484.4
827	TTTCTATGAGTATCTGATCA	971	57.06	0.20	-0.485	0.062	-0.277	~~-0.510~~	502.0
828	TTCTATGAGTATCTGATCAT	972	56.70	-0.50	-0.538	-0.547	-0.541	~~-0.569~~	637.3
829	TCTATGAGTATCTGATCATA	973	55.75	-1.10	-0.678	-1.069	-0.826	~~-0.657~~	489.0
830	CTATGAGTATCTGATCATAC	974	54.95	-1.30	-0.794	-1.243	-0.965	~~-0.712~~	808.7
831	TATGAGTATCTGATCATACT	975	54.95	-1.10	-0.794	-1.069	-0.899	*-0.738*	903.2
832	ATGAGTATCTGATCATACTG	976	55.49	-1.20	-0.715	-1.156	-0.883	~~-0.707~~	1709.3
833	TGAGTATCTGATCATACTGT	977	58.64	-1.20	-0.254	-1.156	-0.597	~~-0.601~~	2103.9
834	GAGTATCTGATCATACTGTC	978	60.20	-1.20	-0.025	-1.156	-0.455	~~-0.468~~	3973.4
835	AGTATCTGATCATACTGTCT	979	60.88	-1.00	0.076	-0.982	-0.326	~~-0.330~~	6462.3
836	GTATCTGATCATACTGTCTT	980	61.03	-0.30	0.097	-0.373	-0.081	~~-0.167~~	9749.0

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
837	TATCTGATCATACTGTCTTA	981	57.16	0.90	-0.470	0.670	-0.037	~~-0.059~~	7817.2
838	ATCTGATCATACTGTCTTAC	982	58.34	0.90	-0.298	0.670	0.070	~~0.007~~	9683.1
839	TCTGATCATACTGTCTTACT	983	60.42	0.90	0.008	0.670	0.259	~~0.055~~	8089.0
840	CTGATCATACTGTCTTACTT	984	59.32	0.90	-0.154	0.670	0.159	~~0.067~~	8696.8
841	TGATCATACTGTCTTACTTT	985	57.63	0.90	-0.401	0.670	0.006	~~0.064~~	6880.5
842	GATCATACTGTCTTACTTTG	986	57.63	0.90	-0.401	0.670	0.006	~~0.020~~	7033.7
843	ATCATACTGTCTTACTTTGA	987	57.63	0.90	-0.401	0.670	0.006	~~-0.093~~	5406.5
844	TCATACTGTCTTACTTTGAT	988	57.63	0.70	-0.401	0.496	-0.060	~~-0.215~~	4239.4
845	CATACTGTCTTACTTTGATA	989	55.68	0.70	-0.688	0.496	-0.238	~~-0.378~~	3727.4
846	ATACTGTCTTACTTTGATAA	990	52.44	0.70	-1.163	0.496	-0.533	~~-0.550~~	2665.5
847	TACTGTCTTACTTTGATAAA	991	50.65	0.70	-1.426	0.496	-0.696	~~-0.696~~	1817.8
848	ACTGTCTTACTTTGATAAAA	992	49.49	-0.30	-1.595	-0.373	-1.131	*-0.809*	1335.9
849	CTGTCTTACTTTGATAAAAC	993	49.49	-0.50	-1.595	-0.547	-1.197	*-0.916*	1526.2
850	TGTCTTACTTTGATAAAACC	994	51.45	-0.50	-1.309	-0.547	-1.019	*-0.949*	822.7
851	GTCTTACTTTGATAAAACCT	995	53.32	-0.50	-1.034	-0.547	-0.849	*-0.966*	1227.4
852	TCTTACTTTGATAAAACCTC	996	51.75	-0.50	-1.264	-0.547	-0.991	*-0.946*	503.0
853	CTTACTTTGATAAAACCTCC	997	54.28	-0.50	-0.894	-0.547	-0.762	*-0.910*	1174.3
854	TTACTTTGATAAAACCTCCA	998	53.70	-0.50	-0.978	-0.547	-0.814	*-0.901*	885.5
855	TACTTTGATAAAACCTCCAA	999	51.79	-0.50	-1.259	-0.547	-0.988	*-0.916*	650.6
856	ACTTTGATAAAACCTCCAAT	1000	52.29	-0.50	-1.185	-0.547	-0.943	*-0.826*	615.4
857	CTTTGATAAAACCTCCAATT	1001	52.11	-0.50	-1.212	-0.547	-0.959	~~-0.728~~	563.4
858	TTTGATAAAACCTCCAATTC	1002	51.46	-0.30	-1.307	-0.373	-0.952	~~-0.561~~	420.9
859	TTGATAAAACCTCCAATTCC	1003	54.68	0.60	-0.834	0.410	-0.362	~~-0.298~~	536.6
860	TGATAAAACCTCCAATTCCC	1004	57.79	0.60	-0.378	0.410	-0.079	~~-0.022~~	1417.8
861	GATAAAACCTCCAATTCCCC	1005	61.15	1.00	0.114	0.757	0.359	~~0.258~~	4351.2
862	ATAAAACCTCCAATTCCCCC	1006	63.24	1.90	0.421	1.540	0.846	~~0.560~~	7738.7
863	TAAAACCTCCAATTCCCCCT	1007	64.88	1.90	0.663	1.540	0.996	~~0.817~~	11136.0

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
864	AAAACCTCCAATTCCCCCTA	1008	64.88	1.90	0.663	1.540	0.996	**1.074**	14811.0
865	AAACCTCCAATTCCCCCTAT	1009	66.73	1.90	0.933	1.540	1.164	**1.261**	15751.0
866	AACCTCCAATTCCCCCTATC	1010	70.07	1.80	1.424	1.453	1.435	**1.330**	19661.0
867	ACCTCCAATTCCCCCTATCA	1011	73.21	1.80	1.883	1.453	1.720	**1.335**	20301.0
868	CCTCCAATTCCCCCTATCAT	1012	72.64	1.80	1.801	1.453	1.669	**1.327**	19376.0
869	CTCCAATTCCCCCTATCATT	1013	69.66	1.60	1.364	1.279	1.332	**1.254**	17642.0
870	TCCAATTCCCCCTATCATTT	1014	68.21	1.10	1.150	0.844	1.034	**1.093**	13751.0
871	CCAATTCCCCCTATCATTTT	1015	67.12	1.10	0.991	0.844	0.935	**0.931**	12669.0
872	CAATTCCCCCTATCATTTTT	1016	64.02	1.10	0.536	0.844	0.653	~~0.818~~	9255.9
873	AATTCCCCCTATCATTTTTG	1017	62.80	0.40	0.357	0.236	0.311	~~0.753~~	8929.1
874	ATTCCCCCTATCATTTTTGG	1018	67.28	0.00	1.014	-0.112	0.586	~~0.715~~	6148.2
875	TTCCCCCTATCATTTTTGGT	1019	70.46	0.00	1.480	-0.112	0.875	~~0.664~~	5468.0
876	TCCCCCTATCATTTTTGGTT	1020	70.46	0.00	1.480	-0.112	0.875	~~0.653~~	5803.7
877	CCCCCTATCATTTTTGGTTT	1021	69.27	0.00	1.307	-0.112	0.768	~~0.658~~	5192.0
878	CCCCTATCATTTTTGGTTTC	1022	67.18	0.00	1.000	-0.112	0.577	~~0.549~~	3557.4
879	CCCTATCATTTTTGGTTTCC	1023	67.18	0.00	1.000	-0.112	0.577	~~0.392~~	5274.3
880	CCTATCATTTTTGGTTTCCA	1024	64.63	0.00	0.625	-0.112	0.345	~~0.270~~	3787.9
881	CTATCATTTTTGGTTTCCAT	1025	60.77	-0.50	0.059	-0.547	-0.171	~~0.167~~	2726.8
882	TATCATTTTTGGTTTCCATC	1026	60.20	-0.50	-0.025	-0.547	-0.223	~~0.092~~	3249.9
883	ATCATTTTTGGTTTCCATCT	1027	62.83	-0.50	0.361	-0.547	0.016	~~0.051~~	5548.9
884	TCATTTTTGGTTTCCATCTT	1028	63.21	-0.50	0.416	-0.547	0.050	~~0.071~~	5290.0
885	CATTTTTGGTTTCCATCTTC	1029	63.21	-0.50	0.416	-0.547	0.050	~~0.157~~	7451.0
886	ATTTTTGGTTTCCATCTTCC	1030	65.88	-0.50	0.809	-0.547	0.293	~~0.262~~	11578.0
887	TTTTTGGTTTCCATCTTCCT	1031	67.93	-0.50	1.109	-0.547	0.480	~~0.366~~	13722.0
888	TTTTGGTTTCCATCTTCCTG	1032	67.42	-0.50	1.035	-0.547	0.434	~~0.475~~	15064.0
889	TTTGGTTTCCATCTTCCTGG	1033	69.71	-0.90	1.370	-0.895	0.509	~~0.554~~	10869.0
890	TTGGTTTCCATCTTCCTGGC	1034	73.74	-1.30	1.962	-1.243	0.744	~~0.535~~	16035.0

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
891	TGGTTTCCATCTTCCTGGCA	1035	74.48	-1.30	2.071	-1.243	0.812	~~0.457~~	16304.0
892	GGTTTCCATCTTCCTGGCAA	1036	72.21	-1.30	1.737	-1.243	0.605	~~0.406~~	14885.0
893	GTTTCCATCTTCCTGGCAAA	1037	67.37	-1.30	1.027	-1.243	0.165	~~0.358~~	11910.0
894	TTTCCATCTTCCTGGCAAAC	1038	64.82	-1.30	0.653	-1.243	-0.067	~~0.290~~	11929.0
895	TTCCATCTTCCTGGCAAACT	1039	66.34	-1.30	0.877	-1.243	0.071	~~0.252~~	11517.0
896	TCCATCTTCCTGGCAAACTC	1040	67.47	-1.30	1.042	-1.243	0.174	~~0.237~~	11822.0
897	CCATCTTCCTGGCAAACTCA	1041	67.12	-0.90	0.991	-0.895	0.274	~~0.285~~	11710.0
898	CATCTTCCTGGCAAACTCAT	1042	63.55	0.90	0.466	0.670	0.544	~~0.357~~	7635.3
899	ATCTTCCTGGCAAACTCATT	1043	62.71	1.00	0.343	0.757	0.501	~~0.409~~	8378.2
900	TCTTCCTGGCAAACTCATTT	1044	63.06	0.90	0.395	0.670	0.500	~~0.446~~	6321.4
901	CTTCCTGGCAAACTCATTTC	1045	63.06	0.70	0.395	0.496	0.434	~~0.468~~	7659.0
902	TTCCTGGCAAACTCATTTCT	1046	63.06	0.70	0.395	0.496	0.434	~~0.429~~	11621.0
903	TCCTGGCAAACTCATTTCTT	1047	63.06	0.70	0.395	0.496	0.434	~~0.363~~	3389.0
904	CCTGGCAAACTCATTTCTTC	1048	63.06	0.70	0.395	0.496	0.434	~~0.273~~	3870.6
905	CTGGCAAACTCATTTCTTCT	1049	61.24	0.70	0.127	0.496	0.268	~~0.160~~	1992.7
906	TGGCAAACTCATTTCTTCTA	1050	58.74	0.70	-0.239	0.496	0.040	~~-0.015~~	698.3
907	GGCAAACTCATTTCTTCTAA	1051	56.86	0.70	-0.514	0.496	-0.130	~~-0.201~~	718.3
908	GCAAACTCATTTCTTCTAAT	1052	54.36	0.70	-0.882	0.496	-0.358	~~-0.339~~	372.3
909	CAAACTCATTTCTTCTAATA	1053	49.93	0.60	-1.530	0.410	-0.793	~~-0.430~~	180.6
910	AAACTCATTTCTTCTAATAC	1054	49.11	0.60	-1.651	0.410	-0.868	~~-0.455~~	430.0
911	AACTCATTTCTTCTAATACT	1055	52.79	0.60	-1.111	0.410	-0.533	~~-0.491~~	904.3
912	ACTCATTTCTTCTAATACTG	1056	54.63	0.60	-0.842	0.410	-0.366	~~-0.510~~	1663.5
913	CTCATTTCTTCTAATACTGT	1057	57.14	0.60	-0.474	0.410	-0.138	~~-0.459~~	2694.2
914	TCATTTCTTCTAATACTGTA	1058	54.51	0.60	-0.859	0.410	-0.377	~~-0.361~~	3222.9
915	CATTTCTTCTAATACTGTAT	1059	53.21	0.60	-1.049	0.410	-0.495	~~-0.310~~	3142.8
916	ATTTCTTCTAATACTGTATC	1060	53.13	0.80	-1.061	0.583	-0.436	~~-0.270~~	5867.0
917	TTTCTTCTAATACTGTATCA	1061	54.51	1.20	-0.859	0.931	-0.179	~~-0.253~~	6641.4

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
918	TTCTTCTAATACTGTATCAT	1062	54.17	1.30	-0.908	1.018	-0.176	~~-0.229~~	7151.9
919	TCTTCTAATACTGTATCATC	1063	55.17	1.30	-0.762	1.018	-0.086	~~-0.139~~	8134.9
920	CTTCTAATACTGTATCATCT	1064	55.86	1.30	-0.661	1.018	-0.023	~~-0.048~~	8551.4
921	TTCTAATACTGTATCATCTG	1065	53.80	1.30	-0.964	1.018	-0.211	~~-0.003~~	5741.7
922	TCTAATACTGTATCATCTGC	1066	57.65	1.30	-0.398	1.018	0.140	~~0.101~~	8575.9
923	CTAATACTGTATCATCTGCT	1067	58.28	1.30	-0.307	1.018	0.197	~~0.248~~	8980.3
924	TAATACTGTATCATCTGCTC	1068	57.65	1.30	-0.398	1.018	0.140	~~0.384~~	10762.0
925	AATACTGTATCATCTGCTCC	1069	62.19	1.30	0.268	1.018	0.553	~~0.566~~	17037.0
926	ATACTGTATCATCTGCTCCT	1070	66.43	1.30	0.889	1.018	0.938	~~0.682~~	20970.0
927	TACTGTATCATCTGCTCCTG	1071	66.32	1.30	0.874	1.018	0.929	~~0.763~~	23084.0
928	ACTGTATCATCTGCTCCTGT	1072	70.36	0.60	1.466	0.410	1.065	**0.875**	24474.0
929	CTGTATCATCTGCTCCTGTA	1073	69.13	0.60	1.286	0.410	0.953	**0.910**	22217.0
930	TGTATCATCTGCTCCTGTAT	1074	67.04	0.60	0.979	0.410	0.763	**0.890**	19829.0
931	GTATCATCTGCTCCTGTATC	1075	68.85	0.60	1.244	0.410	0.927	**0.842**	23548.0
932	TATCATCTGCTCCTGTATCT	1076	67.44	0.60	1.037	0.410	0.799	~~0.773~~	21759.0
933	ATCATCTGCTCCTGTATCTA	1077	67.44	0.60	1.037	0.410	0.799	~~0.725~~	22711.0
934	TCATCTGCTCCTGTATCTAA	1078	65.13	0.60	0.699	0.410	0.589	~~0.706~~	18134.0
935	CATCTGCTCCTGTATCTAAT	1079	63.60	1.00	0.475	0.757	0.582	~~0.611~~	17772.0
936	ATCTGCTCCTGTATCTAATA	1080	61.77	1.60	0.207	1.279	0.614	~~0.502~~	17134.0
937	TCTGCTCCTGTATCTAATAG	1081	62.01	1.60	0.241	1.279	0.635	~~0.389~~	10969.0
938	CTGCTCCTGTATCTAATAGA	1082	61.90	0.50	0.225	0.323	0.262	~~0.336~~	9556.3
939	TGCTCCTGTATCTAATAGAG	1083	60.12	0.30	-0.036	0.149	0.034	~~0.264~~	3739.9
940	GCTCCTGTATCTAATAGAGC	1084	64.50	-1.00	0.607	-0.982	0.003	~~0.187~~	4088.3
941	CTCCTGTATCTAATAGAGCT	1085	62.21	0.30	0.271	0.149	0.224	~~0.106~~	2263.0
942	TCCTGTATCTAATAGAGCTT	1086	60.56	0.30	0.028	0.149	0.074	~~0.080~~	1018.0
943	CCTGTATCTAATAGAGCTTC	1087	60.56	0.30	0.028	0.149	0.074	~~0.091~~	1319.1
944	CTGTATCTAATAGAGCTTCC	1088	60.56	0.30	0.028	0.149	0.074	~~0.070~~	2347.8

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
945	TGTATCTAATAGAGCTTCCT	1089	60.56	0.30	0.028	0.149	0.074	~~0.018~~	1871.6
946	GTATCTAATAGAGCTTCCTT	1090	61.00	0.30	0.092	0.149	0.114	~~-0.010~~	3469.1
947	TATCTAATAGAGCTTCCTTT	1091	58.20	0.30	-0.318	0.149	-0.141	~~-0.030~~	1114.6
948	ATCTAATAGAGCTTCCTTTA	1092	58.20	0.30	-0.318	0.149	-0.141	~~-0.057~~	1358.4
949	TCTAATAGAGCTTCCTTTAG	1093	58.39	0.30	-0.289	0.149	-0.123	~~-0.078~~	665.4
950	CTAATAGAGCTTCCTTTAGT	1094	60.12	0.00	-0.036	-0.112	-0.065	~~-0.019~~	807.4
951	TAATAGAGCTTCCTTTAGTT	1095	58.46	0.30	-0.280	0.149	-0.117	~~0.128~~	608.7
952	AATAGAGCTTCCTTTAGTTG	1096	58.97	0.30	-0.205	0.149	-0.070	~~0.332~~	623.8
953	ATAGAGCTTCCTTTAGTTGC	1097	65.53	0.30	0.758	0.149	0.526	~~0.576~~	674.5
954	TAGAGCTTCCTTTAGTTGCC	1098	69.50	0.30	1.340	0.149	0.887	**0.841**	814.3
955	AGAGCTTCCTTTAGTTGCCC	1099	73.89	0.30	1.983	0.149	1.286	**1.157**	1183.8
956	GAGCTTCCTTTAGTTGCCCC	1100	77.20	0.30	2.470	0.149	1.588	**1.454**	2219.4
957	AGCTTCCTTTAGTTGCCCCC	1101	79.38	0.30	2.789	0.149	1.785	**1.650**	4642.2
958	GCTTCCTTTAGTTGCCCCCC	1102	82.41	0.40	3.234	0.236	2.095	**1.765**	8804.8
959	CTTCCTTTAGTTGCCCCCCT	1103	80.06	0.80	2.889	0.583	2.013	**1.823**	11331.0
960	TTCCTTTAGTTGCCCCCCTA	1104	77.67	1.10	2.539	0.844	1.895	**1.818**	12976.0
961	TCCTTTAGTTGCCCCCCTAT	1105	77.27	0.60	2.480	0.410	1.693	**1.765**	12369.0
962	CCTTTAGTTGCCCCCCTATC	1106	77.27	0.60	2.480	0.410	1.693	**1.669**	15090.0
963	CTTTAGTTGCCCCCCTATCT	1107	75.74	0.60	2.255	0.410	1.554	**1.581**	16130.0
964	TTTAGTTGCCCCCCTATCTT	1108	74.23	0.60	2.033	0.410	1.416	**1.545**	15304.0
965	TTAGTTGCCCCCCTATCTTT	1109	74.23	0.60	2.033	0.410	1.416	**1.539**	14829.0
966	TAGTTGCCCCCCTATCTTTA	1110	73.31	0.80	1.899	0.583	1.399	**1.490**	15309.0
967	AGTTGCCCCCCTATCTTTAT	1111	73.83	1.40	1.976	1.105	1.645	**1.498**	15205.0
968	GTTGCCCCCCTATCTTTATT	1112	73.91	1.40	1.986	1.105	1.652	**1.524**	14192.0
969	TTGCCCCCCTATCTTTATTG	1113	70.59	1.40	1.500	1.105	1.350	**1.515**	8699.5
970	TGCCCCCCTATCTTTATTGT	1114	73.39	1.40	1.911	1.105	1.605	**1.461**	7786.6
971	GCCCCCCTATCTTTATTGTG	1115	73.39	1.40	1.911	1.105	1.605	**1.328**	6709.1

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
972	CCCCCCTATCTTTATTGTGA	1116	70.61	1.40	1.502	1.105	1.351	**1.165**	6198.4
973	CCCCCTATCTTTATTGTGAC	1117	67.66	1.20	1.070	0.931	1.017	**0.999**	4910.2
974	CCCCTATCTTTATTGTGACG	1118	64.37	1.20	0.587	0.931	0.718	~~0.780~~	850.0
975	CCCTATCTTTATTGTGACGA	1119	62.05	1.20	0.248	0.931	0.507	~~0.570~~	404.9
976	CCTATCTTTATTGTGACGAG	1120	58.56	1.20	-0.265	0.931	0.190	~~0.436~~	166.6
977	CTATCTTTATTGTGACGAGG	1121	57.28	1.20	-0.452	0.931	0.073	~~0.376~~	126.9
978	TATCTTTATTGTGACGAGGG	1122	57.91	1.20	-0.361	0.931	0.130	~~0.279~~	92.6
979	ATCTTTATTGTGACGAGGGG	1123	61.03	1.20	0.097	0.931	0.414	~~0.173~~	97.9
980	TCTTTATTGTGACGAGGGGT	1124	64.18	0.90	0.559	0.670	0.601	~~0.097~~	122.3
981	CTTTATTGTGACGAGGGGTC	1125	64.18	-0.80	0.559	-0.808	0.039	~~0.013~~	267.0
982	TTTATTGTGACGAGGGGTCG	1126	62.63	-1.20	0.332	-1.156	-0.233	~~-0.073~~	396.0
983	TTATTGTGACGAGGGGTCGT	1127	65.37	-2.30	0.734	-2.112	-0.348	~~-0.145~~	446.0
984	TATTGTGACGAGGGGTCGTT	1128	65.37	-2.80	0.734	-2.547	-0.513	~~-0.202~~	661.9
985	ATTGTGACGAGGGGTCGTTG	1129	65.82	-2.80	0.800	-2.547	-0.472	~~-0.163~~	864.5
986	TTGTGACGAGGGGTCGTTGC	1130	70.01	-2.80	1.414	-2.547	-0.091	~~-0.156~~	1465.7
987	TGTGACGAGGGGTCGTTGCC	1131	73.21	-2.80	1.884	-2.547	0.200	~~-0.157~~	2836.9
988	GTGACGAGGGGTCGTTGCCA	1132	74.44	-2.80	2.065	-2.547	0.312	~~-0.137~~	3589.7
989	TGACGAGGGGTCGTTGCCAA	1133	69.05	-2.80	1.274	-2.547	-0.178	~~-0.058~~	2100.4
990	GACGAGGGGTCGTTGCCAAA	1134	67.10	-2.80	0.988	-2.547	-0.355	~~0.042~~	1948.7
991	ACGAGGGGTCGTTGCCAAAG	1135	66.13	-2.60	0.845	-2.373	-0.378	~~0.125~~	1384.3
992	CGAGGGGTCGTTGCCAAAGA	1136	66.81	-1.40	0.945	-1.330	0.081	~~0.187~~	1192.0
993	GAGGGGTCGTTGCCAAAGAG	1137	66.84	0.20	0.950	0.062	0.612	~~0.304~~	1221.0
994	AGGGGTCGTTGCCAAAGAGT	1138	68.70	0.20	1.223	0.062	0.782	~~0.427~~	953.2
995	GGGGTCGTTGCCAAAGAGTG	1139	68.32	0.20	1.167	0.062	0.747	~~0.515~~	988.6
996	GGGTCGTTGCCAAAGAGTGA	1140	67.11	0.20	0.989	0.062	0.636	~~0.476~~	937.8
997	GGTCGTTGCCAAAGAGTGAT	1141	64.59	0.50	0.620	0.323	0.507	~~0.333~~	852.1
998	GTCGTTGCCAAAGAGTGATC	1142	63.51	0.00	0.461	-0.112	0.243	~~0.176~~	1189.4

Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA T_m (°C)	ΔG_{MFOLD} (kcal/mole @ 35 °C)	T_m Score	ΔG_{MFOLD} Score	Composite Score	Window-Averaged Composite Score	HIV PRT GeneChip™ Data
999	TCGTTGCCAAAGAGTGATCT	1143	62.35	-1.00	0.291	-0.982	-0.192	-0.012	1501.7
1000	CGTTGCCAAAGAGTGATCTG	1144	60.92	-1.20	0.081	-1.156	-0.389	-0.156	1360.9
1001	GTTGCCAAAGAGTGATCTGA	1145	61.71	-1.20	0.198	-1.156	-0.317	-0.263	1112.9
1002	TTGCCAAAGAGTGATCTGAG	1146	58.90	-1.20	-0.215	-1.156	-0.572	-0.353	468.3
1003	TGCCAAAGAGTGATCTGAGG	1147	61.08	-1.20	0.104	-1.156	-0.375	-0.454	400.1
1004	GCCAAAGAGTGATCTGAGGG	1148	63.68	-1.50	0.485	-1.417	-0.237	-0.541	401.6
1005	CCAAAGAGTGATCTGAGGGA	1149	60.94	-1.20	0.084	-1.156	-0.387	-0.575	199.9
1006	CAAAGAGTGATCTGAGGGAA	1150	55.32	-1.20	-0.741	-1.156	-0.899	-0.530	202.1
1007	AAAGAGTGATCTGAGGGAAG	1151	54.21	-1.20	-0.903	-1.156	-0.999	-0.491	258.7
1008	AAGAGTGATCTGAGGGAAGT	1152	59.12	-1.20	-0.183	-1.156	-0.552	-0.475	274.7
1009	AGAGTGATCTGAGGGAAGTT	1153	61.60	-1.00	0.181	-0.982	-0.261	-0.463	297.2
1010	GAGTGATCTGAGGGAAGTTA	1154	60.78	-0.30	0.061	-0.373	-0.104	-0.414	250.6
1011	AGTGATCTGAGGGAAGTTAA	1155	57.35	0.60	-0.443	0.410	-0.119	-0.318	231.3
1012	GTGATCTGAGGGAAGTTAAA	1156	55.25	0.60	-0.751	0.410	-0.310	-0.286	214.5
1013	TGATCTGAGGGAAGTTAAAG	1157	52.55	0.60	-1.147	0.410	-0.556	-0.295	102.3
1014	GATCTGAGGGAAGTTAAAGG	1158	55.09	0.60	-0.774	0.410	-0.324	-0.330	102.3
1015	ATCTGAGGGAAGTTAAAGGA	1159	55.09	0.60	-0.774	0.410	-0.324	-0.367	49.4
1016	TCTGAGGGAAGTTAAAGGAT	1160	55.09	0.60	-0.774	0.410	-0.324	-0.379	104.3
1017	CTGAGGGAAGTTAAAGGATA	1161	53.32	1.00	-1.034	0.757	-0.353	-0.370	46.3
1018	TGAGGGAAGTTAAAGGATAC	1162	51.95	1.30	-1.235	1.018	-0.378	-0.360	50.9
1019	GAGGGAAGTTAAAGGATACA	1163	53.26	0.90	-1.043	0.670	-0.392		58.2
1020	AGGGAAGTTAAAGGATACAG	1164	52.14	0.90	-1.207	0.670	-0.494		50.5
1021	GGGAAGTTAAAGGATACAGT	1165	54.81	0.90	-0.815	0.670	-0.251		53.1

Example 3

Synopsis: The method of the present invention is particularly useful as a guide to the iterative refinement of probes. One of the specific predictions made for rabbit β-globin in Example 1 is used to provide an example of such a refinement.

Materials and Methods: The contig spanning positions 5-11 of a portion of the rabbit β-globin gene (Example 1, Table 3) was analyzed, using the experimentally measured data to simulate the results of successive experimental measurements. The iterative refinement was performed using a rule-based algorithm, outlined below. This algorithm is used by way of example only; other algorithms for efficiently finding local maxima are well known to the art and could be employed to perform this task.

Given experimental data for probes from the 1st quartile, median and 3rd quartile of a contig, as well as a user-set signal threshold for further consideration of a probe,

1) If all 3 measurements are below the user-specified signal threshold, discard the prediction.
2) If at least one of the measurements is above the user-specified threshold, determine which point yields the maximum signal.
   a) If the maximum point is the 1st quartile probe, then make three new measurements for probes with the same spacing as that used in the preceding iteration, but displaced so that the third probe is identical to the original 1st quartile probe. In other words, repeat the search with the same pattern and spacing, but displace the pattern in the direction of increasing signal found in the first experiment.
   b) If the maximum point is the 3rd quartile probe, then make three new measurements for probes with the same spacing as that used in the preceding iteration, but displaced so that the first probe is identical to the original 3rd quartile probe. In other words, repeat the search with the same

pattern and spacing, but displace the pattern in the direction of increasing signal found in the first experiment.

c) If the maximum point is the median probe, then repeat the experiment, keeping the median point the same, but shrinking the spacing between probes by a factor of 2.

3) Continue iteration until a maximum is found, or the user judges the signal level observed to be acceptable. Use the experimental value measured for the probe duplicated in successive iterations to tie together the successive data sets, via a simple normalization procedure, described below. Where appropriate, consider all of the data (i.e. all of the iterations) when deciding how to proceed, or whether the peak hybridization intensity has been found.

Results: Iterative refinement of the contig spanning positions 5-11 in Table 3 proceeds as follows:

Iteration 1: Probes are synthesized at positions 6, 8 and 10, yielding the experimental hybridization intensities 180, 220 and 310, respectively.

Iteration 2: Following rule 2b), probes are synthesized at positions 10, 12 and 14. Note that the redundant measurement at position 10 serves as a bridge between experiments, and allows comparison of the two sets by normalizing the intensities by multiplying the second iteration measurements by the ratio of the intensity observed for the probe at position 10 in the first iteration to the value observed in the second iteration. In the simplest case, the ratio is 1; in any case, the second iteration yields the normalized values 310, 390, 240 for probe positions 10, 12 and 14, respectively.

Iteration 3: By rule 2c), measurements are performed for probes at positions 11, 12 and 13; after normalization, these yield the normalized hybridization intensities 320, 390 and 410, respectively. Combination of these results with the results from iteration 2, probe position 14, yields the conclusion that the best probe for this intensity peak is the probe that starts at sequence position 13.

The overall result is that iterative improvement converges in three iterations, and requires the synthesis of seven test probes, one of which is the local optimal probe. In addition, the first and second iterations yield probes that exhibit 75% and 95% of the local maximum hybridization intensities, respectively. In many applications, either of these probes would be considered acceptable.

The above examples 1 and 2 demonstrate that two different implementations of the method of the present invention are capable of efficiently predicting regions of high hybridization efficiency in a variety of polynucleotide targets. Many of the predictions yield acceptable probe sequences on the first design iteration, and all would yield optimized probe sets after 2-4 rounds of iterative refinement, as demonstrated in Example 3. The performance demonstrated in these examples greatly exceeds the performance of current methods. Finally, the examples demonstrate that the predictions can be performed by a software application that has been implemented and installed on a Pentium®-based computer workstation.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

```
Attribute VB_Name = "General"
Option Explicit

'Program and DB Rev
Public Const ProgRev$ = "PP Rev 1.00"
Public Const DBRev$ = "PP MDB Template 1.04"

'Columns collection
Public PSColumns As New Collection

'Statistics XArray
Public StatArray As New XArray

'Registry keys
Public Const SKEY_PPPPP$ = "PamelaProbeDesigner"        'application key
Public Const VALUE_DB_TEMPLATE$ = "DBTemplate"          'workspace DB template
Public Const VALUE_DB_DIR$ = "DBDir"                    'workspace DBs directory
Public Const VALUE_DB_FILE_EXT$ = "DBFileExt"           'workspace DB extension
Public Const VALUE_GB_DIR$ = "GBDir"                    'Genbank directory
Public Const VALUE_GB_FILE_EXT$ = "GBFileExt"           'GenBank file extension
Public Const VALUE_BLAST_DIR$ = "BLASTDir"              'BLAST databases
directory

'Error codes
Enum ppErrors
    ppErrGBFileLength = 3000
    ppErrGBFileFormat = 3001
    ppErrBadRecordset = 3002
    ppErrNoProbesCreated = 3003
    ppErrCancelled = 3004
    ppErrDBFormat = 3005
    ppErrBlastVersion = 3006
    ppErrFASTAFormat = 3007
    ppErrSeqLoadDB = 3008
    ppErrDLL = 3009
End Enum

'Flags to prevent sequence and probeset selectors firing.
'Currently broken -- RowColChange events are ignored.
Public AllowdbgClick As Boolean

'Algorithm parameter sets
Public CreatePSPars As New cCreatePSPars
Public PosFilterPars As New cPosFilterPars
Public LengthFilterPars As New cLengthFilterPars
Public GCFilterPars As New cGCFilterPars
Public dGDPars As New cdGDPars
Public dGDFilterPars As New cdGDFilterPars
Public TMPars As New cTMPars
Public TMFilterPars As New cTMFilterPars
Public dGHPars As New cDGHPars
Public dGHFilterPars As New cdGHFilterPars
Public dGMPars As New cdGMPars
Public dGMFilterPars As New cdGMFilterPars
Public RunPars As New cRunPars
Public RunFilterPars As New CRunFilterPars
Public Progress As New cProgress
Public EntrezPars As New cEntrezPars
```



```
Public TrimPars As New cTrimPars
Public ClampPars As New cClampPars
Public ClampFilterPars As New cClampFilterPars
Public BlastPars As New cBLASTPars
Public HomologyPars As New cHomologyPars
Public HomoFilterPars As New cHomoFilterPars
Public ArrayPars As New cArrayPars

' SeqRecord Structure
' This structure holds read from one sequence file/DB/website.
' It it used when reading Genbank files (in which case all fields
' are filled in), or FASTA files (in which case only the Header and
' Sequence portions are filled in).
Public Type SeqRecord
    Header As String
    Locus As String
    Accession As String
    Length As Long
    Sequence As String
End Type

Sub Main()

'Initialize thermodynamic parameters.
InitThermoPars

'Start it up!
frmMain.Show

End Sub
```

```
Attribute VB_Name = "Blast"
Option Explicit

Private Declare Function InitPH Lib "homologydll.dll" _
    (ByVal DB$, ByVal NumProbes&, ByVal W&, ByVal MaxProbeLength&) As Long

Private Declare Function AddProbe Lib "homologydll.dll" _
    (ByVal Probe$, ByVal ProbeLength&) As Long

Private Declare Function CalcOneHomology Lib "homologydll.dll" (ByVal
SeqLength&) As Long

Private Declare Function SkipSequence Lib "homologydll.dll" (ByVal SeqLength&)
As Long

Private Declare Function GetHomology Lib "homologydll.dll" (ByRef Homology&) As
Long

Private Declare Function TerminatePH Lib "homologydll.dll" () As Long

Public Const MaxBLASTHeaders& = 100
```



```
Public Sub CalcHomology(Seq$(), HP As cHomologyPars, Homology&(), RSBlast As
Recordset, PSName$)
'-------------------------------------------------------------------------------
'Function
'   Calculate the homology of an array of probes to a database of sequences.
'Arguments
'   Seq: The sequences.
'   HP: The homology parameters.
'   Homology: The homologies.
'   RSBLast: The table of BLAST-located homologies.
'   PSName: The name of the probeset we are processing.
'-------------------------------------------------------------------------------
On Error GoTo E

Dim NumSeqs&        'number of sequences we are working with
Dim MaxProbeLen&    'longest probe
Dim P&, S#          'index
Dim DBFile&         'header file for database
Dim SR As SeqRecord 'one header from the header file
Dim LineText$       'one line from the header file
Dim NumHeaders&     'number of headers in the header file
Dim DoHomology As Boolean       'is homology to be done for this sequence
Dim foo&            'bit bucket
Dim N&, NumNoCheck&
Dim NoCheck$()      'list of accession numbers not to check homology against
Dim HomologyBaseName            'base name of the DB files needed for homology

'Determine the number of probes we are calculating homology for.
NumSeqs = UBound(Seq) + 1

'Find the longest probe.
MaxProbeLen = 0
For P = 0 To NumSeqs - 1
    If Len(Seq(P)) > MaxProbeLen Then MaxProbeLen = Len(Seq(P))
Next P

'Check for the existence of the homologizer files.
HomologyBaseName = Left(HP.DB, Len(HP.DB) - 4)
foo = FileLen(HP.Path & "\" & HomologyBaseName & ".headers")
foo = FileLen(HP.Path & "\" & HomologyBaseName & ".seqs")

'Initialize the homologizer.
If (InitPH(HP.Path & "\" & HomologyBaseName & ".seqs", NumSeqs, HP.Seed,
MaxProbeLen) <> 0) Then _
    Err.Raise pperrdll, , "Error in InitPH DLL Call."

'Load the probes into the homologizer index.
For P = 0 To NumSeqs - 1
    If (AddProbe(Seq(P), Len(Seq(P))) <> 0) Then _
        Err.Raise pperrdll, , "Error in AddProbe DLL Call."
Next P

'Open the specified DB.
DBFile = FreeFile
Open HP.Path & "\" & HomologyBaseName & ".headers" For Input As #DBFile

'Read the number of entries expected.  The progressbar for this application
'is posted with a maximum of 100, since we don't know the number of sequences
```



```
'in advance of opening the DB.
Line Input #DBFile, LineText
NumHeaders = CLng(LineText)

'Create an array of all accessions that should not be searched against.
NumNoCheck = 0
If IsGoodRS(RSBlast) Then
    RSBlast.MoveLast
    RSBlast.MoveFirst
    NumNoCheck = RSBlast.RecordCount
End If
ReDim NoCheck(0 To NumNoCheck)
N = 0
Do While Not RSBlast.EOF
    If Not RSBlast("Use") Then
        NoCheck(N) = RSBlast("Accession")
        N = N + 1
    End If
    RSBlast.MoveNext
Loop
NumNoCheck = N

'Homologize all the sequences in the DB.
S = 0
Do While Not EOF(DBFile)
    S = S + 1
    If (Fix(100 * S / NumHeaders) - Progress.StopAt > 0) Then
Progress.CheckProgress Fix(100 * S / NumHeaders)
    'Read the next DB sequence.
    SR = ReadFASTAHeader(DBFile)
    'Check the header against the bad list.
    DoHomology = True
    For N = 0 To NumNoCheck - 1
        If SR.Accession = NoCheck(N) Then DoHomology = False
    Next N
    'Do it.
    If DoHomology Then
        If (CalcOneHomology(SR.Length) <> 0) Then _
            Err.Raise pperrdll, , "Error in CalcOneHomology DLL Call."
    Else
        If (SkipSequence(SR.Length) <> 0) Then _
            Err.Raise pperrdll, , "Error in SkipSequence DLL Call."
    End If
Loop

'Retrieve the answers, and clean up.
If (GetHomology(Homology(0)) <> 0) Then _
    Err.Raise pperrdll, , "Error in GetHomology DLL Call."
If (TerminatePH() <> 0) Then _
    Err.Raise pperrdll, , "Error in TerminatePH DLL Call."

Exit Sub
E: Debug.Print "Error in CalcHomology"
Err.Raise Err.Number, , Err.Description
End Sub
```

```
Public Function BLASTPS&(PSName$, ByVal Sequence$, BP As cBLASTPars,
MatchAccession$(), _
    MatchHeader$(), MatchScores#(), MatchExpects#())
'-------------------------------------------------------------------------
'Function
'    Run BLAST on the sequence of a probeset, saving headers and scores of
'    homologues.
'Arguments
'    PSName: The probeset name, used to name temporary files.
'    Sequence: The base sequence of the probeset.
'    BP: The BLAST search parameters.
'    MatchAccession: Matching accession numbers.
'    MatchHeader: The full matching header.
'    MatchScores: The match scores.
'    MatchExpects: The expected number of matches with such scores.
'Notes
'    This is a very fragile function, since it depends on the format of the
'    BLAST output file (checked only for version number), and relies on every
'    header containing an accession number (though one will be manufactured
'    if none is found).
'-------------------------------------------------------------------------
On Error GoTo E

Dim BIFileName$, BOFileName$     'BLAST input and output files.
Dim BIFile&, BOFile&             'BLAST input and output file descriptor.
Dim BLASTBaseName$               'the root name for the BLAST files.
Dim cmd$                         'BLAST command string.
Dim ProcID                       'Process ID of running BLAST program.
Dim foo                          'Bit bucket.
Dim LineText$                    'One line of the BLAST output file.
Dim H&                           'Index the matches.
Dim GBWhere&                     'Location of the GenBank accession within the
match header.

'Check for the existence of all the BLAST files.  If files are absent, an error
will occur.
BLASTBaseName = Left(BP.DB, Len(BP.DB) - 4)
foo = FileLen(BP.Path & "\blastall.exe")
foo = FileLen(BP.Path & "\" & BLASTBaseName & ".nin")
foo = FileLen(BP.Path & "\" & BLASTBaseName & ".nhr")
foo = FileLen(BP.Path & "\" & BLASTBaseName & ".nsq")

'BLAST file names.
BIFileName = PSName & ".fsa"
BOFileName = PSName & ".out"

'Open the BLAST input file.
BIFile = FreeFile
Open BP.Path & "\" & BIFileName For Output As BIFile

'Write out the header.
Print #BIFile, "> PSName = " & PSName

'Write out the sequence.
Do While Len(Sequence) > 60
    Print #BIFile, Mid$(Sequence, 1, 60)
    Sequence = Right(Sequence, Len(Sequence) - 60)
Loop
```



```
Print #BIFile, Sequence

'Close input file.
Close BIFile

'Put together the command string to execute.
BOFileName = PSName & ".out"
cmd = "cmd /c cd " & BP.Path
cmd = cmd & " & blastall -p blastn -i " & BIFileName & " -d " & BLASTBaseName &
" -o " & BOFileName

'Run it and wait.
ProcID = Shell(cmd, vbHide)
foo = WaitOnProgram(ProcID)

'Open the output file.
BOFile = FreeFile
Open BP.Path & "\" & BOFileName For Input As #BOFile

'Check for correct version
Line Input #BOFile, LineText
If Left(LineText, 12) <> "BLASTN 2.0.2" Then Err.Raise ppErrBlastVersion

'Move down to MatchExpects.
On Err GoTo ErrNoSignificantMatch
Do
    Line Input #BOFile, LineText
Loop Until InStr(LineText, "Sequences producing significant alignments:") <> 0
On Error GoTo E

'Strip one more line
Line Input #BOFile, LineText

'Read match information, until a blank line.
Line Input #BOFile, LineText
H = 0
Do
    LineText = Right(LineText, Len(LineText) - 68)
    MatchScores(H) = CDbl(Left(LineText, 4))
    LineText = Right(LineText, Len(LineText) - 4)
    If IsNumeric(LineText) Then
        MatchExpects(H) = CDbl(LineText)
    Else
        MatchExpects(H) = 0#
    End If
    H = H + 1
    Line Input #BOFile, LineText
Loop Until Len(LineText) = 0

'We will return the number of matches.
BLASTPS = H - 1

'Read as many headers as we found matches.
For H = 0 To BLASTPS - 1

    'Strip lines until we see ">".
    Do
        Line Input #BOFile, LineText
```

```
    Loop Until Left(LineText, 1) = ">"

    'Push this line onto the header.
    MatchHeader(H) = LineText

    'Keep pushing on left-justified lines until we see "Length".
    Do
        Line Input #BOFile, LineText
        LineText = LTrim(LineText)
        If (Left(LineText, 6) = "Length") Then Exit Do
        MatchHeader(H) = MatchHeader(H) & " " & LineText
    Loop Until False

    'Find the accession number.
    GBWhere = InStr(MatchHeader(H), "/gb=")
    If (GBWhere = 0) Then
        MatchAccession(H) = "Z00000"
    Else
        GBWhere = GBWhere + 4
        Do
            MatchAccession(H) = MatchAccession(H) & Mid(MatchHeader(H), GBWhere,
1)
            GBWhere = GBWhere + 1
        Loop Until (Mid(MatchHeader(H), GBWhere, 1) = " ")
    End If
Next H

'Remove the BLAST files.
Close #BOFile
Kill BP.Path & "\" & BIFileName
Kill BP.Path & "\" & BOFileName

Exit Function

ErrNoSignificantMatch:
'No match was found, so we've skipped over the match information.
BLASTPS = 0
Close #BOFile
Kill BP.Path & "\" & BIFileName
Kill BP.Path & "\" & BOFileName
Exit Function

BLASTPS = 0
E: Debug.Print "Error in BLASTPS"
Err.Raise Err.Number, , Err.Description
End Function
```

```
Attribute VB_Name = "ColumnValidation"
Option Explicit

Public Sub ColumnsValidate(RSPS As Recordset)
'--------------------------------------------------------------------------------
'Function
'   Check the validity of columns.
'Arguments
'   RSPS: The parameter recordset for the ProbeSets being checked.
'Notes
'   1. A column is valid if the values of the parameters used
'      to calculate it are equal to the values of those parameters
'      in the current instance of the appropriate parameter class.
'   2. This calculation affects settings in the global state variable
'      PSColumns.  It should therefore only be called on the ProbeSets and
'      Probes used to build dbgProbes, i.e. datSelPS.
'   3. Filter columns are always valid, since they are initialized with True.
'--------------------------------------------------------------------------------

Dim CreatePSValid As Boolean
Dim LengthFilterValid As Boolean
Dim PosFilterValid As Boolean
Dim GCFilterValid As Boolean
Dim dGDValid As Boolean
Dim TMValid As Boolean
Dim dGHValid As Boolean
Dim dGMValid As Boolean
Dim ClampValid As Boolean
Dim RunValid As Boolean
Dim HomologyValid As Boolean
Dim Col As cColumn

'On startup, no columns exist, so bail.
If PSColumns.Count = 0 Then Exit Sub

'If the recordsets are empty, then nothing is valid.
If Not IsGoodRS(RSPS) Then
    For Each Col In PSColumns
        If Col.CanBeInValid Then Col.IsValid = False
    Next Col
    Exit Sub
End If

'Set everything valid.
CreatePSValid = True
LengthFilterValid = True
PosFilterValid = True
GCFilterValid = True
dGDValid = True
TMValid = True
dGHValid = True
dGMValid = True
RunValid = True
ClampValid = True
HomologyValid = True

'Try to invalidate.
RSPS.MoveFirst
Do While Not RSPS.EOF
    CreatePSValid = CreatePSValid And CreatePSPars.Validate(RSPS)
    LengthFilterValid = LengthFilterValid And LengthFilterPars.Validate(RSPS)
    PosFilterValid = PosFilterValid And PosFilterPars.Validate(RSPS)
    GCFilterValid = GCFilterValid And GCFilterPars.Validate(RSPS)
    dGDValid = dGDValid And dGDPars.Validate(RSPS) And
dGDFilterPars.Validate(RSPS)
    TMValid = TMValid And TMPars.Validate(RSPS) And TMFilterPars.Validate(RSPS)
    dGHValid = dGHValid And dGHPars.Validate(RSPS) And
dGHFilterPars.Validate(RSPS)
```

```
    dGMValid = dGMValid And dGMPars.Validate(RSPS) And
dGMFilterPars.Validate(RSPS)
    RunValid = RunValid And RunPars.Validate(RSPS) And
RunFilterPars.Validate(RSPS)
    ClampValid = ClampValid And ClampPars.Validate(RSPS) And
ClampFilterPars.Validate(RSPS)
    HomologyValid = HomologyValid And HomologyPars.Validate(RSPS) And
HomoFilterPars.Validate(RSPS)
    RSPS.MoveNext
Loop

'Update columns.
PSColumns("Sequence").IsValid = CreatePSValid
PSColumns("Length").IsValid = CreatePSValid And LengthFilterValid
PSColumns("Length Filter").IsValid = PSColumns("Length").IsValid
PSColumns("Position").IsValid = CreatePSValid And PosFilterValid
PSColumns("Pos Filter").IsValid = PSColumns("Position").IsValid
PSColumns("GC").IsValid = CreatePSValid And GCFilterValid
PSColumns("GC Filter").IsValid = PSColumns("GC").IsValid
PSColumns("Duplex dG").IsValid = dGDValid
PSColumns("dGD Filter").IsValid = PSColumns("Duplex dG").IsValid
PSColumns("TM").IsValid = TMValid
PSColumns("TM Filter").IsValid = PSColumns("TM").IsValid
PSColumns("dGH").IsValid = dGHValid
PSColumns("dGH Filter").IsValid = PSColumns("dGH").IsValid
PSColumns("dGM").IsValid = dGMValid
PSColumns("dGM Filter").IsValid = PSColumns("dGM").IsValid
PSColumns("Run Length").IsValid = RunValid
PSColumns("Run Filter").IsValid = PSColumns("Run Length").IsValid
PSColumns("Clamp").IsValid = ClampValid
PSColumns("Clamp Filter").IsValid = PSColumns("Clamp").IsValid
PSColumns("Homology").IsValid = HomologyValid
PSColumns("Homology Filter").IsValid = PSColumns("Homology").IsValid

'Set column heading backcolor according to validity.
For Each Col In PSColumns
    If Col.CanBeInValid Then
        If Col.IsValid Then
            frmMain.dbgProbes.Columns(Col.Name).HeadForeColor = vbBlack
        Else
            frmMain.dbgProbes.Columns(Col.Name).HeadForeColor = vbRed
        End If
    End If
Next Col

End Sub
Public Sub ColumnsExist(RSPS As Recordset, RSProbes As Recordset)
'--------------------------------------------------------------------------------
'Function
'   Check the existence of columns.
'Arguments
'   RSPS: The probeset(s) parameter recordset.
'   PSProbes: The probeset probes.
'Notes
'   1. A column exists if
'      A. For each probeset, the appropriate existence parameter is set.
'         This indicates that some calculation has been performed to fill in
'         the column, at sometime in the past.  However, it is possible that
```



```
'         the selection criteria have changed since the calculation, bringing
'         into scope probes that have not had the calculation performed.  Thus,
'         the existence parameter is necessary, but not sufficient.
'      B. For each probe, the appropriate value is not NULL.   A lack of
'         null values is necessary and sufficient; however, it is potentially
'         time-consuming to check, so condition A is checked first, and
'         condition B is checked only if A passes.
'   2. This calculation affects settings in the global state variable
'      PSColumns.  It should therefore only be called on the ProbeSets and
'      Probes used to build dbgProbes, i.e. datSelPS and datSelPQuery.
'   3. Filter columns always exist, since they are initialized with True.
'--------------------------------------------------------------------------------

Dim Exists As Boolean
Dim Col As cColumn

'On startup, no columns exist, so bail.
If PSColumns.Count = 0 Then Exit Sub

'If the recordsets are empty, then nothing exists, nothing is valid.
If Not (IsGoodRS(RSPS) And IsGoodRS(RSProbes)) Then
    For Each Col In PSColumns
        If Col.CanUnExist Then Col.Exists = False
    Next Col
    Exit Sub
End If

'Probe creation calculations.
Exists = True
RSPS.MoveFirst
Do While Not RSPS.EOF
    Exists = Exists And RSPS("CreatePS-Exists")
    RSPS.MoveNext
Loop
PSColumns("Accession").Exists = Exists
PSColumns("PSName").Exists = Exists
PSColumns("Sequence").Exists = Exists
PSColumns("Length").Exists = Exists
PSColumns("Position").Exists = Exists
PSColumns("GC").Exists = Exists

'TM Calculations.
Exists = True
RSPS.MoveFirst
Do While Not RSPS.EOF
    Exists = Exists And RSPS("TM-Exists")
    RSPS.MoveNext
Loop
If Exists = True Then
    RSProbes.FindFirst "TM = NULL"
    If Not RSProbes.NoMatch Then Exists = False
End If
PSColumns("TM").Exists = Exists

'dGH Calculations.
Exists = True
RSPS.MoveFirst
Do While Not RSPS.EOF
```

```
    Exists = Exists And RSPS("dGH-Exists")
    RSPS.MoveNext
Loop
If Exists = True Then
    RSProbes.FindFirst "dGH = NULL"
    If Not RSProbes.NoMatch Then Exists = False
End If
PSColumns("dGH").Exists = Exists

'dGM Calculations.
Exists = True
RSPS.MoveFirst
Do While Not RSPS.EOF
    Exists = Exists And RSPS("dGM-Exists")
    RSPS.MoveNext
Loop
If Exists = True Then
    RSProbes.FindFirst "dGM = NULL"
    If Not RSProbes.NoMatch Then Exists = False
End If
PSColumns("dGM").Exists = Exists

'Clamp Calculations.
Exists = True
RSPS.MoveFirst
Do While Not RSPS.EOF
    Exists = Exists And RSPS("Clamp-Exists")
    RSPS.MoveNext
Loop
If Exists = True Then
    RSProbes.FindFirst "[Clamp] = NULL"
    If Not RSProbes.NoMatch Then Exists = False
End If
PSColumns("Clamp").Exists = Exists

'Run Length Calculations.
Exists = True
RSPS.MoveFirst
Do While Not RSPS.EOF
    Exists = Exists And RSPS("Run-Exists")
    RSPS.MoveNext
Loop
If Exists = True Then
    RSProbes.FindFirst "[Run Length] = NULL"
    If Not RSProbes.NoMatch Then Exists = False
End If
PSColumns("Run Length").Exists = Exists

'Homology Calculations.
Exists = True
RSPS.MoveFirst
Do While Not RSPS.EOF
    Exists = Exists And RSPS("Homology-Exists")
    RSPS.MoveNext
Loop
If Exists = True Then
    RSProbes.FindFirst "[Homology] = NULL"
    If Not RSProbe.NoMatch Then Exists = False
```



```
End If
PSColumns("Homology").Exists = Exists

End Sub
```

```
Attribute VB_Name = "CreateProbeSet"
Option Explicit

Public Function CreatePSEqualL&(Seq$, CPSP As cCreatePSPars, Probes$(), _
    Positions&(), GC#())
'-------------------------------------------------------------------------------
'Function:
'   Create a set of probes using the EqualLength method.
'Parameters:
'   Seq: The sequence from which the probes are created.
'   CPSP: An instance of the parameter class for this algorithm.
'   Probes: The array in which the probes will be returned.
'   Positions: The array in which the starting positions will be returned.
'   GC: The array in which GC content will be returned.
'Returns:
'   The number of probes created.
'Notes:
'   2. The calling routine is responsible for adequately dimensioning Probes,
'      Positions, and GC.
'-------------------------------------------------------------------------------

Dim StartPos&     'the starting position of a probe
Dim ProbeNum&     'the current probe

StartPos = 1
ProbeNum = LBound(Probes)
Do While StartPos <= Len(Seq) - CPSP.Length + 1
    If ProbeNum - Progress.StopAt = 0 Then Progress.CheckProgress StartPos
    Probes(ProbeNum) = Mid(Seq, StartPos, CPSP.Length)
    Positions(ProbeNum) = StartPos
    GC(ProbeNum) = DNA_GCContent(Probes(ProbeNum))
    StartPos = StartPos + CPSP.Spacing
    ProbeNum = ProbeNum + 1
Loop
CreatePSEqualL = ProbeNum - 1
End Function

Public Function CreatePSEqualTM&(Seq$, CPSP As cCreatePSPars, Probes$(), _
    Positions&(), GC#())
'-------------------------------------------------------------------------------
'Function:
'   Create a set of probes using the EqualTM method.
'Parameters:
'   Seq: The sequence from which the probes are created.
'   CPSP: An instance of the parameter class for this algorithm.
'   Probes: The array in which the probes will be returned.
'   Positions: The array in which the starting positions will be returned.
'   GC: The array in which GC content will be returned.
'Returns:
'   The number of probes created.
'Notes:
'   2. The calling routine is responsible for adequately dimensioning Probes,
```



```
'     Positions, and GC.
'-------------------------------------------------------------------------

Dim StartPos&     'the starting position of a probe
Dim ProbeNum&     'the current probe
Dim Length&       'current length of the probe being created
Dim TM#           'current TM of the probe being created

StartPos = 1
ProbeNum = LBound(Probes)
Do While StartPos <= Len(Seq)
    'Check progress
    If ProbeNum - Progress.StopAt = 0 Then Progress.CheckProgress StartPos
    'Create the probes, extending until melting point is above target.
    Length = 1
    Probes(ProbeNum) = Mid(Seq, StartPos, Length)
    Select Case CPSP.Duplex
    Case "DNA/DNA"
        TM = DNA_CalcTM(Probes(ProbeNum), CPSP.Conc)
    Case "DNA/RNA"
        TM = DR_CalcTM(Probes(ProbeNum), CPSP.Conc)
    End Select
    Length = Length + 1
    Do While TM < CPSP.Target
        If StartPos + Length > Len(Seq) Then
            ProbeNum = ProbeNum - 1
            Exit Do
        End If
        Probes(ProbeNum) = Mid(Seq, StartPos, Length)
        Select Case CPSP.Duplex
        Case "DNA/DNA"
            TM = DNA_CalcTM(Probes(ProbeNum), CPSP.Conc)
        Case "DNA/RNA"
            TM = DR_CalcTM(Probes(ProbeNum), CPSP.Conc)
        End Select
        Length = Length + 1
    Loop
    Positions(ProbeNum) = StartPos
    GC(ProbeNum) = DNA_GCContent(Probes(ProbeNum))
    ProbeNum = ProbeNum + 1
    StartPos = StartPos + CPSP.Spacing
Loop
CreatePSEqualTM = ProbeNum - 1
End Function
```

```
Attribute VB_Name = "DNA_Manipulations"
Option Explicit

Public Function DNA_GCContent#(ByVal Seq$)
'-------------------------------------------------------------------------
'Function
'   Compute the GC content of a sequence.
'-------------------------------------------------------------------------
Dim i&
Seq = LCase(Seq)
DNA_GCContent = 0
For i = 1 To Len(Seq)
```



```
    If (Mid(Seq, i, 1) = "g" Or Mid(Seq, i, 1) = "c") Then DNA_GCContent =
DNA_GCContent + 1
Next i
DNA_GCContent = DNA_GCContent / Len(Seq) * 100
End Function

Public Function DNA_SeqTrim$(Seq$, Bases&, WhichEnd$)
'-------------------------------------------------------------------------
'Function
'   Trim off one end or the other of the sequence.
'-------------------------------------------------------------------------
If Bases < Len(Seq) Then
    If WhichEnd = "5'" Then DNA_SeqTrim = Right(Seq, Len(Seq) - Bases)
    If WhichEnd = "3'" Then DNA_SeqTrim = Left(Seq, Len(Seq) - Bases)
End If
End Function

Public Function DNA_SeqKeep$(Seq$, Bases&, WhichEnd$)
'-------------------------------------------------------------------------
'Function
'   Remove all but the specified number of bases.
'-------------------------------------------------------------------------
If Bases <= Len(Seq) Then
    If WhichEnd = "5'" Then DNA_SeqKeep = Left(Seq, Bases)
    If WhichEnd = "3'" Then DNA_SeqKeep = Right(Seq, Bases)
End If
End Function

Public Function DNA_RevComp$(ByVal Seq$)
'-------------------------------------------------------------------------
'Function
'   Given 5'-3' strand, returns 5'-3' complement.
'Revision
'   28-Jul-97: From PkWs routine of the same name.
'-------------------------------------------------------------------------
Dim i&
Seq = LCase(Seq)
For i = Len(Seq) To 1 Step -1
    Select Case Mid$(Seq, i, 1)
    Case "a"
        DNA_RevComp = DNA_RevComp & "t"
    Case "c"
        DNA_RevComp = DNA_RevComp & "g"
    Case "g"
        DNA_RevComp = DNA_RevComp & "c"
    Case "t"
        DNA_RevComp = DNA_RevComp & "a"
    Case "u"
        DNA_RevComp = DNA_RevComp & "a"
    Case "n"
        DNA_RevComp = DNA_RevComp & "n"
    Case Else
        MsgBox "Unknown base found while calculating DNA_RevComp."
        DNA_RevComp = Seq
        Exit Function
    End Select
Next i
End Function
```

```
Public Function DNA_Comp$(ByVal Seq$)
'-------------------------------------------------------------------------------
'Function
'   Given 5'-3' strand, returns complement.  This might be useless!
'-------------------------------------------------------------------------------
Dim i&
Seq = LCase(Seq)
For i = 1 To Len(Seq)
    Select Case Mid$(Seq, i, 1)
    Case "a"
        DNA_Comp = DNA_Comp & "t"
    Case "c"
        DNA_Comp = DNA_Comp & "g"
    Case "g"
        DNA_Comp = DNA_Comp & "c"
    Case "t"
        DNA_Comp = DNA_Comp & "a"
    Case "u"
        DNA_Comp = DNA_Comp & "a"
    Case "n"
        DNA_Comp = DNA_Comp & "n"
    Case Else
        MsgBox "Unknown base found while calculating DNA_Comp."
        DNA_Comp = Seq
        Exit Function
    End Select
Next i
End Function

Public Function DNA_Rev$(ByVal Seq$)
'-------------------------------------------------------------------------------
'Function
'   Given 5'-3' strand, returns 3'-5' sequence.  This might be useless!
'-------------------------------------------------------------------------------
Dim i&
Seq = LCase(Seq)
For i = Len(Seq) To 1 Step -1
    Select Case Mid$(Seq, i, 1)
    Case "a"
        DNA_Rev = DNA_Rev & "a"
    Case "c"
        DNA_Rev = DNA_Rev & "c"
    Case "g"
        DNA_Rev = DNA_Rev & "g"
    Case "t"
        DNA_Rev = DNA_Rev & "t"
    Case "u"
        DNA_Rev = DNA_Rev & "u"
    Case "n"
        DNA_Rev = DNA_Rev & "n"
    Case Else
        MsgBox "Unknown base found while calculating DNA_Rev."
        DNA_Rev = Seq
        Exit Function
    End Select
Next i
End Function
```



```
Public Sub DNA_Str2Num(ByVal Seq$, ByRef NumSeq%())
'-------------------------------------------------------------------------------
'Function
'   Return a numeric array representing the DNA string.
'Arguments
'   Seq: The original sequence.
'   NumSeq: An integer array representing the sequence, w/ lower bound 0.
'Notes:
'   1. NumSeq must be correctly dimensioned by the calling routine.
'   since VB won't let me return an array from a function.
'-------------------------------------------------------------------------------
Dim i&
Seq = LCase(Seq)
For i = 0 To Len(Seq) - 1
    Select Case Mid(Seq, i + 1, 1)
    Case "a"
        NumSeq(i) = 0
    Case "c"
        NumSeq(i) = 1
    Case "g"
        NumSeq(i) = 2
    Case "t"
        NumSeq(i) = 3
    Case "u"
        NumSeq(i) = 3
    Case "n"
        NumSeq(i) = -1
    Case Else
        MsgBox "Unknown base found while calculating DNA_Str2Num."
    End Select
Next i
End Sub

Public Function DNA_Num2Str$(NumSeq%())
'-------------------------------------------------------------------------------
'Function:
'   Return the string representation of the DNA string
'Arguments:
'   NumSeq: An integer array representing the sequence.
'-------------------------------------------------------------------------------
Dim i&
For i = 0 To UBound(NumSeq)
    Select Case NumSeq(i)
    Case "0"
        DNA_Num2Str = DNA_Num2Str & "a"
    Case "1"
        DNA_Num2Str = DNA_Num2Str & "c"
    Case "2"
        DNA_Num2Str = DNA_Num2Str & "g"
    Case "3"
        DNA_Num2Str = DNA_Num2Str & "t"
    Case "-1"
        DNA_Num2Str = DNA_Num2Str & "n"
    Case Else
        MsgBox "Unknown numeric code found while calculating DNA_Num2Str."
    End Select
Next i
```



```
End Function
```

```
Attribute VB_Name = "Engine"
Option Explicit

Private Sub PSCalcBlast(RSPS As Recordset, RSBlast As Recordset)
'--------------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for BLAST calculation.
'Arguments
'   RSPS: The parameters recordset (set parameters used in current record).
'--------------------------------------------------------------------------------
On Error GoTo E

Dim PSName$                             'probe set name.
Dim Sequence$                           'sequence!
Dim MatchAccession$(MaxBLASTHeaders)
Dim MatchHeaders$(MaxBLASTHeaders)      'headers of matches
Dim MatchScores#(MaxBLASTHeaders)       'scores of matches
Dim MatchExpects#(MaxBLASTHeaders)      'expected value of matches
Dim H&                                  'loop index
Dim NumMatches&                         'number of matches found
Dim FindStr$

'Get the probe set name and sequence.
PSName = RSPS("PSName")
Sequence = RSPS("CreatePS-Sequence")
If Len(Sequence) = 0 Then GoTo UpdateOnly

'Run it.
Progress.ShowProgress "Calculating BLAST matches for ProbeSet " & PSName, 0, 1
NumMatches = BLASTPS(PSName, Sequence, BlastPars, MatchAccession, MatchHeaders,
MatchScores, MatchExpects)

'Update the results.
For H = 0 To NumMatches - 1
    With RSBlast
        'Check for this record already being present.
        .FindFirst "PSName = '" & PSName & "' AND Accession = '" &
MatchAccession(H) & "'"
        If .NoMatch Then
            .AddNew
        Else
            .Edit
        End If
        .Fields("PSName") = PSName
        .Fields("Accession") = MatchAccession(H)
        .Fields("Header") = MatchHeaders(H)
        .Fields("Score") = MatchScores(H)
        .Fields("Expect") = MatchExpects(H)
        If MatchScores(H) > BlastPars.SCutoff Or MatchExpects(H) <
BlastPars.ECutoff Then
            .Fields("Use") = False
        Else
            .Fields("Use") = True
        End If
        .Update
```



```
    End With
Next H

UpdateOnly:

'Store parameters in the database.
BlastPars.StoreDB RSPS

Exit Sub
E: Debug.Print "Error in PSCalcBlast"
Err.Raise Err.Number, , Err.Description
End Sub

Private Sub SeqCalcCreatePS(RSSeq As Recordset, RSProbes As Recordset, _
    RSPS As Recordset)
'--------------------------------------------------------------------------------
'Function
'   Create a ProbeSet
'Arguments
'   RSSeq: The sequence recordset (positioned to current sequence).
'   RSPS: The ProbeSets recordset (used to add a new ProbeSet).
'   RSProbes: The Probes recordset (used to add new probes).
'   RSPS: The Parameters recordset (used to add a new parameter record).
'Errors Raised
'   ppErrNoProbesCreated:  Raised if no probes are generated.
'Errors Handled
'   None.
'Notes
'   1. Under normal use (e.g. when called by the GUI), this routine uses the
'   values in CreatePSPars.
'   2. Only RSSeq must be a good recordset, since all the others are
'   used only to add records.
'--------------------------------------------------------------------------------

Dim Accession$          'current accession
Dim Seq$                'current sequence
Dim SeqLength&          'current sequence length
Dim NumPS&              'number of ProbeSets for this sequence
Dim PSName$             'new ProbeSet name
Dim Probes$()           'temporary store for created probes
Dim Positions&()        'temporary store for created positions
Dim GC#()               'temporary store for GC content
Dim NumProbes&          'number of probes created
Dim P&                  'index variable for traversing Probes
Dim ProgressStr$        'text for progress bar

On Error GoTo E

'Check the recordset
If Not IsGoodRS(RSSeq) Then Exit Sub

'Get the sequence, accession, etc.
Accession = RSSeq("Accession")
Seq = RSSeq("Sequence")
SeqLength = RSSeq("Length")
NumPS = RSSeq("NumPS")

'New probeset name.
```

```
PSName = Accession & "_PS" & CStr(NumPS)

'Create temporary store for new probes.
ReDim Probes(SeqLength)
ReDim Positions(SeqLength)
ReDim GC(SeqLength)

'Open the progress bar.
Progress.ShowProgress "Creating ProbeSet " & PSName, 0, NumProbes

'Create the probes.
Select Case CreatePSPars.Method
Case "EqualL"
    NumProbes = CreatePSEqualL(Seq, CreatePSPars, Probes, Positions, GC)
Case "EqualTM"
    NumProbes = CreatePSEqualTM(Seq, CreatePSPars, Probes, Positions, GC)
End Select
If NumProbes = 0 Then Err.Raise ppErrNoProbesCreated
ReDim Preserve Probes(NumProbes)
ReDim Preserve Positions(NumProbes)
ReDim Preserve GC(NumProbes)

'Add a new ProbeSet.
RSSeq.Edit
RSSeq.Fields("NumPS") = NumPS + 1
RSSeq.Update
CreatePSPars.AddNewDB RSPS, Accession, PSName, SeqLength, Seq

'Create new records in the Probes table.
Progress.ShowProgress "Updating Probes in Database for Probeset " & PSName, 0,
NumProbes
For P = 0 To UBound(Probes) - 1
    If P - Progress.StopAt = 0 Then Progress.CheckProgress P
    RSProbes.AddNew
    RSProbes.Fields("Accession") = Accession
    RSProbes.Fields("PSName") = PSName
    RSProbes.Fields("Sequence") = Probes(P)
    RSProbes.Fields("Length") = Len(Probes(P))
    RSProbes.Fields("Position") = Positions(P)
    RSProbes.Fields("GC") = GC(P)
    RSProbes.Update
Next P

'Clean exit.
Exit Sub
E: Debug.Print "Error in SeqCalcCreatePS"
Err.Raise Err.Number, , Err.Description
End Sub

Public Sub SeqCalcEngine(Calc$)
'------------------------------------------------------------------------------
'Function
'   SeqCalcEngine acts as the dispatcher for calculations
'   performed on Sequences.  Each of the known calculations
'   can be requested by specifying its name and subname.
'   SeqCalcEngine then retrieves necessary records from the
'   DB, then calls the specified calculation for each probe.
'Arguments
```



```
'   Calc: The calculation to perform.
'Notes
'   1. The parameters used for a particular calculation are
'      either made available in the optional parameter array
'      Pars, or retrieved from the class properties.
'   2. This routine loops over all selected Sequences.
'Errors
'   Errors are raised by not handled.  Calling routine is responsible
'------------------------------------------------------------------------------

Dim RSSelSeqs As Recordset          'holds all selected Sequences
Dim RSP As Recordset                'holds all probes.
Dim RSAllProbes As Recordset        'holds all probes in one ProbeSet
Dim RSPS As Recordset               'holds parameters for the current ProbeSet
Dim PSName$                         'current ProbeSet name
Dim ErrNumber                       'saves number of errors raised by callee's
Dim ErrDescription                  'save description of errors raised by
callee's

'Error handling.
On Error GoTo E

'Check that some sequences are available.
Set RSSelSeqs = frmMain.datSelSeqs.Recordset
If Not IsGoodRS(RSSelSeqs) Then Exit Sub

'Map recordset pointers.
Set RSP = frmMain.datP.Recordset
Set RSPS = frmMain.datPS.Recordset

'Close the grid.
frmMain.dbgProbes.Close

'Process all selected sequences.
RSSelSeqs.MoveFirst
Do While Not RSSelSeqs.EOF

    'Begin a transaction block
    'XXXXX -- causes an error when entering this routine for the second time, no
idea why.
    'BeginTrans

    Select Case Calc

    Case "CreatePS"

        'Create the new probeset.
        SeqCalcCreatePS RSSelSeqs, RSP, RSPS

        'Get PS Name.
        PSName = RSPS("PSName")

        'Access all probes in this ProbeSet.
        With frmMain.datPOnePS
            .RecordSource = "SELECT * FROM Probes WHERE PSName = '" & PSName &
"'"
            .Refresh
            Set RSAllProbes = .Recordset
```

```
        End With

        'Compute length and position filters.
        If LengthFilterPars.Method <> "None" Then
            PSCalcLengthFilter RSAllProbes, RSPS
            PSColumns("Length Filter").IsFilter = LengthFilterPars.AppImmediate
        End If
        If PosFilterPars.Method <> "None" Then
            PSCalcPosFilter RSAllProbes, RSPS
            PSColumns("Pos Filter").IsFilter = PosFilterPars.AppImmediate
        End If
        If GCFilterPars.Method <> "None" Then
            PSCalcGCFilter RSAllProbes, RSPS
            PSColumns("GC Filter").IsFilter = GCFilterPars.AppImmediate
        End If
        PSColumns("PSName").IsVisible = True
        PSColumns("Sequence").IsVisible = True
        PSColumns("Length").IsVisible = True
        PSColumns("Position").IsVisible = True
        PSColumns("GC").IsVisible = True

    Case "3' Trim"
        SeqCalcTrim frmMain.datSelSeqs.Recordset, "3'"

    Case "5' Trim"
        SeqCalcTrim frmMain.datSelSeqs.Recordset, "5'"

    Case "3' Keep"
        SeqCalcKeep frmMain.datSelSeqs.Recordset, "3'"

    Case "5' Keep"
        SeqCalcKeep frmMain.datSelSeqs.Recordset, "5'"

    End Select

    'End the transaction.
    'CommitTrans

    'Move to next selected sequence.
    RSSelSeqs.MoveNext

Loop

'Update appearances.
frmMain.Form_ChangeSequence
frmMain.Form_ChangePSName

'Reopen the grid.
frmMain.dbgProbes.ReOpen
Progress.Hide

'Clean exit.
Exit Sub

'Handle errors.
E: Debug.Print "Error in SeqCalcEngine."
ErrNumber = Err.Number
ErrDescription = Err.Description
```



```
'Rollback
frmMain.Form_ChangeSequence
frmMain.Form_ChangePSName
frmMain.dbgProbes.ReOpen
Progress.Hide
frmMain.MousePointer = vbDefault
Err.Raise ErrNumber, , ErrDescription

End Sub


Private Sub SeqCalcTrim(RS As Recordset, WhichEnd$)
'-------------------------------------------------------------------------------
'Function
'   Trim bases from the 3' or 5' ends.
'-------------------------------------------------------------------------------
If Not IsGoodRS(RS) Then Exit Sub
RS.Edit
If WhichEnd = "5'" Then
    RS("Sequence") = DNA_SeqTrim(RS("sequence"), TrimPars.FivePTrim, "5'")
Else
    RS("Sequence") = DNA_SeqTrim(RS("sequence"), TrimPars.ThreePTrim, "3'")
End If
RS("Length") = Len(RS("Sequence"))
RS("Accession") = RS("Accession") & "*"
RS.Update
End Sub

Private Sub SeqCalcKeep(RS As Recordset, WhichEnd$)
'-------------------------------------------------------------------------------
'Function
'   Keep bases on the 3' or 5' ends.
'-------------------------------------------------------------------------------
If Not IsGoodRS(RS) Then Exit Sub
RS.Edit
If WhichEnd = "5'" Then
    RS("Sequence") = DNA_SeqKeep(RS("sequence"), TrimPars.FivePKeep, "5'")
Else
    RS("Sequence") = DNA_SeqKeep(RS("sequence"), TrimPars.ThreePKeep, "3'")
End If
RS("Length") = Len(RS("Sequence"))
RS("Accession") = RS("Accession") & "*"
RS.Update
End Sub

Private Sub SetField(RS As Recordset, Field$, Value)
'-------------------------------------------------------------------------------
'Function
'   Set all entries for one field in the recordset.
'-------------------------------------------------------------------------------
RS.MoveFirst
Do While Not RS.EOF
    RS.Edit
    RS.Fields(Field) = Value
    RS.Update
    RS.MoveNext
Loop
End Sub
```

```
Private Sub PutField(RS As Recordset, Field$, Value)
'--------------------------------------------------------------------------------
'Function
'   Put all entries for one field in the recordset.
'Notes
'   Since this operation can be timeconsuming, the progressbar is updated.
'   So a progressbar has to be posted first!
'--------------------------------------------------------------------------------
Dim P&
P = 0
RS.MoveFirst
Do While Not RS.EOF
    If P - Progress.StopAt = 0 Then Progress.CheckProgress P
    RS.Edit
    RS.Fields(Field) = Value(P)
    RS.Update
    P = P + 1
    RS.MoveNext
Loop
End Sub

Private Sub GetField(RS As Recordset, Field$, Values)
'--------------------------------------------------------------------------------
'Function
'   Get all entries for one field in the recordset.
'--------------------------------------------------------------------------------
Dim P&
P = 0
RS.MoveFirst
Do While Not RS.EOF
    Values(P) = RS.Fields(Field)
    RS.MoveNext
    P = P + 1
Loop
End Sub
Private Sub PSCalcdGH(RSProbes As Recordset, RSPS As Recordset)
'--------------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for dGH calculation.
'Arguments
'   RSProbes: The probes recordset (calculate dGH for all records).
'   RSPS: The parameters recordset (set parameters used in current record).
'--------------------------------------------------------------------------------
On Error GoTo E

Dim PSName$             'name of current probeset
Dim NumProbes&          'number of probes in the recordset
Dim Seq$()              'sequence column from database.
Dim dGH#()              'dGH column, to be put to database.

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly

'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating dGH for ProbeSet " & PSName, 0, NumProbes
```



```
'Create space for database fields.
ReDim Seq(0 To NumProbes - 1)
ReDim dGH(0 To NumProbes - 1)

'Get the sequences.
GetField RSProbes, "Sequence", Seq

'Calculate dGH.
Select Case dGHPars.DR
Case "DNA"
    DNA_CalcdGH Seq, dGHPars, dGH
Case "RNA"
    RNA_CalcdGH Seq, dGHPars, dGH
End Select

'Update the results.
Progress.ShowProgress "Updating dGH in Database for ProbeSet " & PSName, 0,
NumProbes
PutField RSProbes, "dGH", dGH

UpdateOnly:

'Store parameters in the database, and update column visibility.
dGHPars.StoreDB RSPS
PSColumns("dGH").Exists = True
PSColumns("dGH").IsVisible = True

'Calculate filter
PSCalcdGHFilter RSProbes, RSPS

Exit Sub
E: Debug.Print "Error in PSCalcdGH"
Err.Raise Err.Number, , Err.Description
End Sub


Private Sub PSCalcdGM(RSProbes As Recordset, RSPS As Recordset)
'--------------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for dGM calculation.
'Arguments
'   RSProbes: The probes recordset (calculate dGM for all records).
'   RSPS: The parameters recordset (set parameters used in current record).
'--------------------------------------------------------------------------------
On Error GoTo E

Dim PSName$             'name of current probeset
Dim NumProbes&          'number of probes in the recordset
Dim Seq$()              'sequence column from database.
Dim dGM#()              'dGM column, to be put to database.

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly

'Start it up.
PSName = RSProbes("PSName")
```

```
Progress.ShowProgress "Calculating dGM for ProbeSet " & PSName, 0, NumProbes

'Create space for database fields.
ReDim Seq(0 To NumProbes - 1)
ReDim dGM(0 To NumProbes - 1)

'Get the sequences.
GetField RSProbes, "Sequence", Seq

'Calculate dGM.
Select Case dGMPars.DR
Case "DNA"
    DNA_CalcdGM Seq, dGMPars, dGM
Case "RNA"
    RNA_CalcdGM Seq, dGMPars, dGM
End Select

'Update the results.
Progress.ShowProgress "Updating dGM in Database for ProbeSet " & PSName, 0,
NumProbes
PutField RSProbes, "dGM", dGM

UpdateOnly:

'Store parameters in the database, and update column visibility.
dGMPars.StoreDB RSPS
PSColumns("dGM").Exists = True
PSColumns("dGM").IsVisible = True

'Calculate filter.
PSCalcdGMFilter RSProbes, RSPS

Exit Sub
E: Debug.Print "Error in PSCalcdGM."
Err.Raise Err.Number, , Err.Description
End Sub


Private Sub PSCalcRun(RSProbes As Recordset, RSPS As Recordset)
'-------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for Run calculation.
'Arguments
'   RSProbes: The probes recordset (calculate Run for all records).
'   RSPS: The parameters recordset (set parameters used in current record).
'-------------------------------------------------------------------------
On Error GoTo E

Dim PSName$             'name of current probeset
Dim NumProbes&          'number of probes in the recordset
Dim Pos&()              'position column from database.
Dim Run&()              'run column, to be put to database.

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly
```



```
'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating Run for ProbeSet " & PSName, 0, NumProbes

'Create space for database fields.
ReDim Pos(0 To NumProbes - 1)
ReDim Run(0 To NumProbes - 1)

'Get the sequences.
GetField RSProbes, "Position", Pos

'Calculate Run.
CalcRun Pos, RunPars, Run

'Update the results.
Progress.ShowProgress "Updating Run in Database for ProbeSet " & PSName, 0,
NumProbes
PutField RSProbes, "Run Length", Run

UpdateOnly:

'Store parameters in the database, and update column visibility.
RunPars.StoreDB RSPS
PSColumns("Run Length").Exists = True
PSColumns("Run Length").IsVisible = True

'Calculate the run filter.
PSCalcRunFilter RSProbes, RSPS

Exit Sub
E: Debug.Print "Error in PSCalcRun"
Err.Raise Err.Number, , Err.Description
End Sub


Private Sub PSCalcHomology(RSProbes As Recordset, RSPS As Recordset, RSBlast As
Recordset)
'-------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for Homology calculation.
'Arguments
'   RSProbes: The probes recordset (calculate Homology for all records).
'   RSPS: The parameters recordset (set parameters used in current record).
'-------------------------------------------------------------------------
On Error GoTo E

Dim PSName$             'name of current probeset
Dim NumProbes&          'number of probes in the recordset
Dim Seq$()              'sequences of probes
Dim Homology&()         'run column, to be put to database

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly

'Start it up.
'Note that the progress bar in this case will show progress over sequences
'in the database.  Since we don't know the total, we'll set the maximum to
```

```
'100, and have the called routine do fraction calculations.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating Homology for ProbeSet " & PSName, 0, 100

'Create space for database fields.
ReDim Seq(0 To NumProbes - 1)
ReDim Homology(0 To NumProbes - 1)

'Get the sequences.
GetField RSProbes, "Sequence", Seq

'Calculate Homology.
CalcHomology Seq, HomologyPars, Homology, RSBlast, PSName

'Update the results.
Progress.ShowProgress "Updating Homology in Database for ProbeSet " & PSName, 0,
NumProbes
PutField RSProbes, "Homology", Homology

UpdateOnly:

'Store parameters in the database, and update column visibility.
HomologyPars.StoreDB RSPS
PSColumns("Homology").Exists = True
PSColumns("Homology").IsVisible = True

'Calculate the homology filter.
PSCalcHomoFilter RSProbes, RSPS

Exit Sub
E: Debug.Print "Error in PSCalcHomology"
Err.Raise Err.Number, , Err.Description
End Sub

Private Sub PSCalcTM(RSProbes As Recordset, RSPS As Recordset)
'-------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for TM calculation.
'Arguments
'   RSProbes: The probes recordset (calculate TM for all records).
'   RSPS: The parameters recordset (set parameters used in current record).
'-------------------------------------------------------------------------
On Error GoTo E

Dim PSName$             'name of current probeset
Dim NumProbes&          'number of probes in the recordset
Dim Seq$()              'sequence column from database.
Dim TM#()               'TM column, to be put to database.

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly

'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating TM for ProbeSet " & PSName, 0, NumProbes

'Create space for database fields.
```



```
ReDim Seq(0 To NumProbes - 1)
ReDim TM(0 To NumProbes - 1)

'Get the sequences.
GetField RSProbes, "Sequence", Seq

'Calculate TM.
Select Case TMPars.Duplex
Case "DNA/DNA"
    DNA_CalcAllTM Seq, TMPars, TM
Case "DNA/RNA"
    DR_CalcAllTM Seq, TMPars, TM
End Select

'Update the results.
Progress.ShowProgress "Updating TM in Database for ProbeSet " & PSName, 0,
NumProbes
PutField RSProbes, "TM", TM

UpdateOnly:

'Store parameters in the database, and update column visibility.
TMPars.StoreDB RSPS
PSColumns("TM").Exists = True
PSColumns("TM").IsVisible = True

'Calculate the filter.
PSCalcTMFilter RSProbes, RSPS

Exit Sub
E: Debug.Print "Error in PSCalcTM"
Err.Raise Err.Number, , Err.Description
End Sub

Private Sub PSCalcdGD(RSProbes As Recordset, RSPS As Recordset)
'-------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for dGD calculation.
'Arguments
'   RSProbes: The probes recordset (calculate dGD for all records).
'   RSPS: The parameters recordset (set parameters used in current record).
'-------------------------------------------------------------------------
On Error GoTo E

Dim PSName$             'name of current probeset
Dim NumProbes&          'number of probes in the recordset
Dim Seq$()              'sequence column from database.
Dim dGD#()              'dGD column, to be put to database.

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly

'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating dG (Duplex) for ProbeSet " & PSName, 0,
NumProbes
```

```
'Create space for database fields.
ReDim Seq(0 To NumProbes - 1)
ReDim dGD(0 To NumProbes - 1)

'Get the sequences.
GetField RSProbes, "Sequence", Seq

'Calculate dGD.
Select Case dGDPars.Duplex
Case "DNA"
    DNA_CalcdGD Seq, dGDPars, dGD
Case "RNA"
    DR_CalcdGD Seq, dGDPars, dGD
End Select

'Update the results.
Progress.ShowProgress "Updating dG (Duplex) in Database for ProbeSet " & PSName,
0, NumProbes
PutField RSProbes, "Duplex dG", dGD

UpdateOnly:

'Store parameters in the database, and update column visibility.
dGDPars.StoreDB RSPS
PSColumns("Duplex dG").Exists = True
PSColumns("Duplex dG").IsVisible = True

'Calculate the filter.
PSCalcdGDFilter RSProbes, RSPS

Exit Sub
E: Debug.Print "Error in PSCalcdGD"
Err.Raise Err.Number, , Err.Description
End Sub


Private Sub PSCalcClamp(RSProbes As Recordset, RSPS As Recordset)
'--------------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for Clamp calculation.
'Arguments
'   RSProbes: The probes recordset (calculate Clamp for all records).
'   RSPS: The parameters recordset (set parameters used in current record).
'--------------------------------------------------------------------------------
On Error GoTo E

Dim PSName$             'name of current probeset
Dim NumProbes&          'number of probes in the recordset
Dim Seq$()              'sequence column from database.
Dim Clamp#()              'Clamp column, to be put to database.

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly

'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating Clamp for ProbeSet " & PSName, 0, NumProbes
```



```
'Create space for database fields.
ReDim Seq(0 To NumProbes - 1)
ReDim Clamp(0 To NumProbes - 1)

'Get the sequences.
GetField RSProbes, "Sequence", Seq

'Calculate Clamp.
Select Case ClampPars.Duplex
Case "DNA/DNA"
    DNA_CalcClamp Seq, ClampPars, Clamp
Case "DNA/RNA"
    'XXXXX
    DNA_CalcClamp Seq, ClampPars, Clamp
End Select

'Update the results.
Progress.ShowProgress "Updating Clamp in Database for ProbeSet " & PSName, 0,
NumProbes
PutField RSProbes, "Clamp", Clamp

UpdateOnly:

'Store parameters in the database, and update column visibility.
ClampPars.StoreDB RSPS
PSColumns("Clamp").Exists = True
PSColumns("Clamp").IsVisible = True

'Calculate the filter
PSCalcClampFilter RSProbes, RSPS

Exit Sub
E: Debug.Print "Error in PSCalcClamp"
Err.Raise Err.Number, , Err.Description
End Sub


Public Sub PSCalcEngine(Calc$)
'--------------------------------------------------------------------------------
'Function
'   PSCalcEngine acts as the dispatcher for calculations
'   performed on ProbeSets.  Each of the known calculations
'   can be requested by specifying its name.
'Arguments
'   Calc: The calculation to perform.
'Notes
'   1. All numeric calculations proceed by
'       - checking that the sequences column exists.
'       - checking that the current parameters set for this calculation
'         are the same as those previously used, and, if not, nulling
'         out all previous calculations.
'       - performing the calculation.
'   2. Filter calculations proceed as above, with the preliminary step
'   of checking the existence of the column to be filtered.  If it isn't
'   there, the calculation is done.
'   3. The Probes grid is closed before DB updates, then opened
'   when they are all over, to reduce screen thrashing.
```

```
'   4. Updates to the database are placed in transaction blocks, so
'   that they can be cleanly cancelled.  This should also improve
'   performance on network drives.
'   5. Calculation is always done one ProbeSet at a time, to reduce, whenever
'   possible, recalculation of values.  This could probably be further improved
'   by only recalculating NULL values (since any non-NULL is guarenteed good by
'   the above checks).
'Errors:
'   Errors are handled by rolling back pending DB transactions, cleaning up the
'   UI, then re-raising the same error for the caller to deal with.
'-------------------------------------------------------------------------

Dim RSSelPS As Recordset            'holds all selected ProbeSets
Dim RSAllProbes As Recordset        'holds all probes in one ProbeSet
Dim RSQProbes As Recordset          'holds probes in one ProbeSet that pass the
filters
Dim RSPS As Recordset               'holds parameters for the current ProbeSet
Dim RSBlast As Recordset            'holds BLAST homologies
Dim PSName$                         'current ProbeSet name
Dim ErrNumber                       'saves number of errors raised by callee's
Dim ErrDescription                  'save description of errors raised by
callee's

'Error handling.
On Error GoTo E

'Check that some ProbeSets are available, with sequence information.
Set RSSelPS = frmMain.datSelPS.Recordset
If Not IsGoodRS(RSSelPS) Then Exit Sub
If Not PSColumns("Sequence").Exists Then Exit Sub
Set RSBlast = frmEditBLAST.datBLAST.Recordset

'Close the grid.
frmMain.MousePointer = vbHourglass
frmMain.dbgProbes.Close

'Post the initial progress bar.
Progress.ShowProgress "Beginning Calculation: " & Calc, 0, 100

'Process all selected ProbeSets.
RSSelPS.MoveFirst
Do While Not RSSelPS.EOF

    'Get PS Name.
    PSName = RSSelPS("PSName")

    'Access all probes in this ProbeSet.
    With frmMain.datPOnePS
        .RecordSource = "SELECT * FROM Probes WHERE PSName = '" & PSName & "'"
        .Refresh
        Set RSAllProbes = .Recordset
    End With

    'Access probes in this ProbeSet that pass the filters.
    With frmMain.datPOnePSQuery
        .RecordSource = BuildPOnePSQuery(PSName)
        .Refresh
        Set RSQProbes = .Recordset
```



```
    End With

    'Position the parameter recordset for updates.
    With frmMain.datPS
        .Recordset.FindFirst "PSName = '" & PSName & "'"
        Set RSPS = .Recordset
    End With

    'Start a transaction block.
    BeginTrans

    'Choose the operation
    Select Case Calc

    Case "dGD"
        If dGDPars.Exists(RSPS) And Not dGDPars.Validate(RSPS) Then SetField
RSAllProbes, "dGD", Null
        If Not dGDFilterPars.Validate(RSPS) Then SetField RSAllProbes, "dGD
Filter", True
        PSCalcdGD RSQProbes, RSPS

    Case "dGD Filter"
        If Not PSColumns("dGD").Exists Then
            If dGDPars.Exists(RSPS) And Not dGDPars.Validate(RSPS) Then SetField
RSAllProbes, "dGD", Null
            If Not dGDFilterPars.Validate(RSPS) Then SetField RSAllProbes, "dGD
Filter", True
            PSCalcdGD RSQProbes, RSPS
        Else
            If Not dGDFilterPars.Validate(RSPS) Then SetField RSAllProbes, "dGD
Filter", True
            PSCalcdGDFilter RSQProbes, RSPS
        End If

    Case "TM"
        If TMPars.Exists(RSPS) And Not TMPars.Validate(RSPS) Then SetField
RSAllProbes, "TM", Null
        If Not TMFilterPars.Validate(RSPS) Then SetField RSAllProbes, "TM
Filter", True
        PSCalcTM RSQProbes, RSPS

    Case "TM Filter"
        If Not PSColumns("TM").Exists Then
            If TMPars.Exists(RSPS) And Not TMPars.Validate(RSPS) Then SetField
RSAllProbes, "TM", Null
            If Not TMFilterPars.Validate(RSPS) Then SetField RSAllProbes, "TM
Filter", True
            PSCalcTM RSQProbes, RSPS
        Else
            If Not TMFilterPars.Validate(RSPS) Then SetField RSAllProbes, "TM
Filter", True
            PSCalcTMFilter RSQProbes, RSPS
        End If

    Case "dGH"
        If dGHPars.Exists(RSPS) And Not dGHPars.Validate(RSPS) Then SetField
RSAllProbes, "dGH", Null
```

```
        If Not dGHFilterPars.Validate(RSPS) Then SetField RSAllProbes, "dGH
Filter", True
        PSCalcdGH RSQProbes, RSPS

    Case "dGH Filter"
        If Not PSColumns("dGH").Exists Then
            If dGHPars.Exists(RSPS) And Not dGHPars.Validate(RSPS) Then SetField
RSAllProbes, "dGH", True
            If Not dGHFilterPars.Validate(RSPS) Then SetField RSAllProbes, "dGH
Filter", True
            PSCalcdGH RSQProbes, RSPS
        Else
            If Not dGHFilterPars.Validate(RSPS) Then SetField RSAllProbes, "dGH
Filter", True
            PSCalcdGHFilter RSQProbes, RSPS
        End If

    Case "dGM"
        If dGMPars.Exists(RSPS) And Not dGMPars.Validate(RSPS) Then SetField
RSAllProbes, "dGM", Null
        If Not dGMFilterPars.Validate(RSPS) Then SetField RSAllProbes, "dGM
Filter", True
        PSCalcdGM RSQProbes, RSPS

    Case "dGM Filter"
        If Not PSColumns("dGM").Exists Then
            If dGMPars.Exists(RSPS) And Not dGMPars.Validate(RSPS) Then SetField
RSAllProbes, "dGM", True
            If Not dGMFilterPars.Validate(RSPS) Then SetField RSAllProbes, "dGM
Filter", True
            PSCalcdGM RSQProbes, RSPS
        Else
            If Not dGMFilterPars.Validate(RSPS) Then SetField RSAllProbes, "dGM
Filter", True
            PSCalcdGMFilter RSQProbes, RSPS
        End If

    Case "Run"
        If RunPars.Exists(RSPS) And Not RunPars.Validate(RSPS) Then SetField
RSAllProbes, "Run Length", Null
        If Not RunFilterPars.Validate(RSPS) Then SetField RSAllProbes, "Run
Filter", True
        PSCalcRun RSQProbes, RSPS

    Case "Run Filter"
        If Not PSColumns("Run Length").Exists Then
            If RunPars.Exists(RSPS) And Not RunPars.Validate(RSPS) Then SetField
RSAllProbes, "Run", True
            If Not RunFilterPars.Validate(RSPS) Then SetField RSAllProbes, "Run
Filter", True
            PSCalcRun RSQProbes, RSPS
        Else
            If Not RunFilterPars.Validate(RSPS) Then SetField RSAllProbes, "Run
Filter", True
            PSCalcRunFilter RSQProbes, RSPS
        End If

    Case "Clamp"
```



```
        If ClampPars.Exists(RSPS) And Not ClampPars.Validate(RSPS) Then SetField
RSAllProbes, "Clamp", Null
        If Not ClampFilterPars.Validate(RSPS) Then SetField RSAllProbes, "Clamp
Filter", True
        PSCalcClamp RSQProbes, RSPS

    Case "Clamp Filter"
        If Not PSColumns("Clamp").Exists Then
            If ClampPars.Exists(RSPS) And Not ClampPars.Validate(RSPS) Then
SetField RSAllProbes, "Clamp", Null
            If Not ClampFilterPars.Validate(RSPS) Then SetField RSAllProbes,
"Clamp Filter", True
            PSCalcClamp RSQProbes, RSPS
        Else
            If Not ClampFilterPars.Validate(RSPS) Then SetField RSAllProbes,
"Clamp Filter", True
            PSCalcClampFilter RSQProbes, RSPS
        End If

    Case "Length Filter"
        If Not PSColumns("Length").Exists Then Exit Sub
        If Not LengthFilterPars.Validate(RSPS) Then SetField RSAllProbes,
"Length Filter", True
        PSCalcLengthFilter RSQProbes, RSPS

    Case "Pos Filter"
        If Not PSColumns("Position").Exists Then Exit Sub
        If Not PosFilterPars.Validate(RSPS) Then SetField RSAllProbes, "Pos
Filter", True
        PSCalcPosFilter RSQProbes, RSPS

    Case "GC Filter"
        If Not PSColumns("GC").Exists Then Exit Sub
        If Not GCFilterPars.Validate(RSPS) Then SetField RSAllProbes, "GC
Filter", True
        PSCalcGCFilter RSQProbes, RSPS

    Case "BLAST"
        PSCalcBlast RSPS, RSBlast

    Case "Homology"
        If HomologyPars.Exists(RSPS) And Not HomologyPars.Validate(RSPS) Then
SetField RSAllProbes, "Homology", Null
        If Not HomoFilterPars.Validate(RSPS) Then SetField RSAllProbes,
"Homology Filter", True
        PSCalcHomology RSQProbes, RSPS, RSBlast

    Case "Homology Filter"
        If Not PSColumns("Homology").Exists Then
            If HomologyPars.Exists(RSPS) And Not HomologyPars.Validate(RSPS)
Then SetField RSAllProbes, "Homology", Null
            If Not HomoFilterPars.Validate(RSPS) Then SetField RSAllProbes,
"Homology Filter", True
            PSCalcHomology RSQProbes, RSPS, RSBlast
        Else
            If Not HomoFilterPars.Validate(RSPS) Then SetField RSAllProbes,
"Homology Filter", True
            PSCalcHomoFilter RSQProbes, RSPS
```



```
        End If

    End Select

    'End the transaction.
    CommitTrans

    'Move to next selected probeset.
    RSSelPS.MoveNext

Loop

'Reopen the grid.
Progress.Hide
frmMain.dbgProbes.ReOpen
DoEvents

'Update appearances.
frmMain.Form_ChangePSName

'Clean exit.
frmMain.MousePointer = vbDefault
Exit Sub

'Handle errors.
E: Debug.Print "Error in PSCalcEngine."
ErrNumber = Err.Number
ErrDescription = Err.Description
Rollback
frmMain.Form_ChangePSName
frmMain.dbgProbes.ReOpen
Progress.Hide
frmMain.MousePointer = vbDefault
Err.Raise ErrNumber, , ErrDescription

End Sub


Private Sub PSCalcTMFilter(RSProbes As Recordset, RSPS As Recordset)
'-------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for TM filter calculation.
'Arguments
'   RSProbes: The probes recordset (calculate TM Filter for all records).
'   RSPS: The parameter recordset (set parameters used in current record).
'-------------------------------------------------------------------------
On Error GoTo E

Dim PSName$                     'name of current probeset
Dim NumProbes&                  'number of probes in the recordset
Dim TM#()                       'TM column from database
Dim TMFilter() As Boolean       'TM Filter column to database

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly
```



```
'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating TM Filter for ProbeSet " & PSName, 0,
NumProbes

'Create space for database fields.
ReDim TM(0 To NumProbes - 1)
ReDim TMFilter(0 To NumProbes - 1)

'Get the TMs.
GetField RSProbes, "TM", TM

'Calculate TM Filter.
CalcTMFilter TM, TMFilterPars, TMFilter

'Update the results.
Progress.ShowProgress "Updating TM Filter in Database for ProbeSet " & PSName,
0, NumProbes
PutField RSProbes, "TM Filter", TMFilter

UpdateOnly:

'Store parameters in the database, and update column visibility.
TMFilterPars.StoreDB RSPS
PSColumns("TM Filter").IsFilter = TMFilterPars.AppImmediate

Exit Sub
E: Debug.Print "Error in PSCalcTMFilter"
Err.Raise Err.Number, , Err.Description
End Sub

Private Sub PSCalcdGDFilter(RSProbes As Recordset, RSPS As Recordset)
'-------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for dGD filter calculation.
'Arguments
'   RSProbes: The probes recordset (calculate dGD Filter for all records).
'   RSPS: The parameter recordset (set parameters used in current record).
'-------------------------------------------------------------------------
On Error GoTo E

Dim PSName$                     'name of current probeset
Dim NumProbes&                  'number of probes in the recordset
Dim dGD#()                      'dGD column from database
Dim dGDFilter() As Boolean      'dGD Filter column to database

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly

'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating dG (Duplex) Filter for ProbeSet " & PSName,
0, NumProbes

'Create space for database fields.
ReDim dGD(0 To NumProbes - 1)
ReDim dGDFilter(0 To NumProbes - 1)
```

```
'Get the dGDs.
GetField RSProbes, "Duplex dG", dGD

'Calculate dGD Filter.
CalcdGDFilter dGD, dGDFilterPars, dGDFilter

'Update the results.
Progress.ShowProgress "Updating dG (Duplex) Filter in Database for ProbeSet " &
PSName, 0, NumProbes
PutField RSProbes, "dGD Filter", dGDFilter

UpdateOnly:

'Store parameters in the database, and update column visibility.
dGDFilterPars.StoreDB RSPS
PSColumns("dGD Filter").IsFilter = dGDFilterPars.AppImmediate

Exit Sub
E: Debug.Print "Error in PSCalcdGDFilter"
Err.Raise Err.Number, , Err.Description
End Sub


Private Sub PSCalcClampFilter(RSProbes As Recordset, RSPS As Recordset)
'-------------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for Clamp filter calculation.
'Arguments
'   RSProbes: The probes recordset (calculate Clamp Filter for all records).
'   RSPS: The parameter recordset (set parameters used in current record).
'-------------------------------------------------------------------------------
On Error GoTo E

Dim PSName$                     'name of current probeset
Dim NumProbes&                  'number of probes in the recordset
Dim Clamp#()                      'Clamp column from database
Dim ClampFilter() As Boolean    'Clamp Filter column to database

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly

'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating Clamp Filter for ProbeSet " & PSName, 0,
NumProbes

'Create space for database fields.
ReDim Clamp(0 To NumProbes - 1)
ReDim ClampFilter(0 To NumProbes - 1)

'Get the Clamp values.
GetField RSProbes, "Clamp", Clamp

'Calculate Clamp Filter.
CalcClampFilter Clamp, ClampFilterPars, ClampFilter
```



```
'Update the results.
Progress.ShowProgress "Updating Clamp Filter in Database for ProbeSet " &
PSName, 0, NumProbes
PutField RSProbes, "Clamp Filter", ClampFilter

UpdateOnly:

'Store parameters in the database, and update column visibility.
ClampFilterPars.StoreDB RSPS
PSColumns("Clamp Filter").IsFilter = ClampFilterPars.AppImmediate

Exit Sub
E: Debug.Print "Error in PSCalcClampFilter"
Err.Raise Err.Number, , Err.Description
End Sub
Private Sub PSCalcdGHFilter(RSProbes As Recordset, RSPS As Recordset)
'-------------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for dGH filter calculation.
'Arguments
'   RSProbes: The probes recordset (calculate dGH Filter for all records).
'   RSPS: The parameter recordset (set parameters used in current record).
'-------------------------------------------------------------------------------
On Error GoTo E

Dim PSName$                     'name of current probeset
Dim NumProbes&                  'number of probes in the recordset
Dim dGH#()                      'dGH column from database
Dim dGHFilter() As Boolean      'dGH Filter column to database

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly

'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating dGH Filter for ProbeSet " & PSName, 0,
NumProbes

'Create space for database fields.
ReDim dGH(0 To NumProbes - 1)
ReDim dGHFilter(0 To NumProbes - 1)

'Get the dGHs.
GetField RSProbes, "dGH", dGH

'Calculate dGH Filter.
CalcdGHFilter dGH, dGHFilterPars, dGHFilter

'Update the results.
Progress.ShowProgress "Updating dGH Filter in Database for ProbeSet " & PSName,
0, NumProbes
PutField RSProbes, "dGH Filter", dGHFilter

UpdateOnly:

'Store parameters in the database, and update column visibility.
dGHFilterPars.StoreDB RSPS
```



```
PSColumns("dGH Filter").IsFilter = dGHFilterPars.AppImmediate

Exit Sub
E: Debug.Print "Error in PSCalcdGHFilter"
Err.Raise Err.Number, , Err.Description
End Sub

Private Sub PSCalcGCFilter(RSProbes As Recordset, RSPS As Recordset)
'-------------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for GC filter calculation.
'Arguments
'   RSProbes: The probes recordset (calculate GC Filter for all records).
'   RSPS: The parameter recordset (set parameters used in current record).
'-------------------------------------------------------------------------------
On Error GoTo E

Dim PSName$                 'name of current probeset
Dim NumProbes&              'number of probes in the recordset
Dim GC#()                   'GC column from database
Dim GCFilter() As Boolean   'GC Filter column to database

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly

'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating GC Filter for ProbeSet " & PSName, 0,
NumProbes

'Create space for database fields.
ReDim GC(0 To NumProbes - 1)
ReDim GCFilter(0 To NumProbes - 1)

'Get the GCs.
GetField RSProbes, "GC", GC

'Calculate GC Filter.
CalcGCFilter GC, GCFilterPars, GCFilter

'Update the results.
Progress.ShowProgress "Updating GC Filter in Database for ProbeSet " & PSName,
0, NumProbes
PutField RSProbes, "GC Filter", GCFilter

UpdateOnly:

'Store parameters in the database, and update column visibility.
GCFilterPars.StoreDB RSPS
PSColumns("GC Filter").IsFilter = GCFilterPars.AppImmediate

Exit Sub
E: Debug.Print "Error in PSCalcGCFilter"
Err.Raise Err.Number, , Err.Description
End Sub

Private Sub PSCalcdGMFilter(RSProbes As Recordset, RSPS As Recordset)
```



```
'-------------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for dGM filter calculation.
'Arguments
'   RSProbes: The probes recordset (calculate dGM Filter for all records).
'   RSPS: The parameter recordset (set parameters used in current record).
'-------------------------------------------------------------------------------
On Error GoTo E

Dim PSName$                 'name of current probeset
Dim NumProbes&              'number of probes in the recordset
Dim dGM#()                  'dGM column from database
Dim dGMFilter() As Boolean  'dGM Filter column to database

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly

'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating dGM Filter for ProbeSet " & PSName, 0,
NumProbes

'Create space for database fields.
ReDim dGM(0 To NumProbes - 1)
ReDim dGMFilter(0 To NumProbes - 1)

'Get the dGMs.
GetField RSProbes, "dGM", dGM

'Calculate dGM Filter.
CalcdGMFilter dGM, dGMFilterPars, dGMFilter

'Update the results.
Progress.ShowProgress "Updating dGM Filter in Database for ProbeSet " & PSName,
0, NumProbes
PutField RSProbes, "dGM Filter", dGMFilter

UpdateOnly:

'Store parameters in the database, and update column visibility.
dGMFilterPars.StoreDB RSPS
PSColumns("dGM Filter").IsFilter = dGMFilterPars.AppImmediate

Exit Sub
E: Debug.Print "Error in PSCalcdGMFilter"
Err.Raise Err.Number, , Err.Description
End Sub

Private Sub PSCalcRunFilter(RSProbes As Recordset, RSPS As Recordset)
'-------------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for Run filter calculation.
'Arguments
'   RSProbes: The probes recordset (calculate Run Filter for all records).
'   RSPS: The parameter recordset (set parameters used in current record).
'-------------------------------------------------------------------------------
On Error GoTo E
```



```
Dim PSName$                     'name of current probeset
Dim NumProbes&                  'number of probes in the recordset
Dim Run&()                      'Run column from database
Dim RunFilter() As Boolean      'Run Filter column to database

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly

'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating Run Filter for ProbeSet " & PSName, 0,
NumProbes

'Create space for database fields.
ReDim Run(0 To NumProbes - 1)
ReDim RunFilter(0 To NumProbes - 1)

'Get the Runs.
GetField RSProbes, "Run Length", Run

'Calculate Run Filter.
CalcRunFilter Run, RunFilterPars, RunFilter

'Update the results.
Progress.ShowProgress "Updating Run Filter in Database for ProbeSet " & PSName,
0, NumProbes
PutField RSProbes, "Run Filter", RunFilter

UpdateOnly:

'Store parameters in the database, and update column visibility.
RunFilterPars.StoreDB RSPS
PSColumns("Run Filter").IsFilter = RunFilterPars.AppImmediate

Exit Sub
E: Debug.Print "Error in PSCalcRunFilter"
Err.Raise Err.Number, , Err.Description
End Sub

Private Sub PSCalcHomoFilter(RSProbes As Recordset, RSPS As Recordset)
'-------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for Homology filter calculation.
'Arguments
'   RSProbes: The probes recordset (calculate Homology Filter for all records).
'   RSPS: The parameter recordset (set parameters used in current record).
'-------------------------------------------------------------------------
On Error GoTo E

Dim PSName$                     'name of current probeset
Dim NumProbes&                  'number of probes in the recordset
Dim Homology&()                     'Homology column from database
Dim HomoFilter() As Boolean     'Homology Filter column to database

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
```



```
If NumProbes = 0 Then GoTo UpdateOnly

'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating Homology Filter for ProbeSet " & PSName, 0,
NumProbes

'Create space for database fields.
ReDim Homology(0 To NumProbes - 1)
ReDim HomoFilter(0 To NumProbes - 1)

'Get the Homologys.
GetField RSProbes, "Homology", Homology

'Calculate Homology Filter.
CalcHomoFilter Homology, HomoFilterPars, HomoFilter

'Update the results.
Progress.ShowProgress "Updating Homology Filter in Database for ProbeSet " &
PSName, 0, NumProbes
PutField RSProbes, "Homology Filter", HomoFilter

UpdateOnly:

'Store parameters in the database, and update column visibility.
HomoFilterPars.StoreDB RSPS
PSColumns("Homology Filter").IsFilter = HomoFilterPars.AppImmediate

Exit Sub
E: Debug.Print "Error in PSCalcHomoFilter"
Err.Raise Err.Number, , Err.Description
End Sub


Private Sub PSCalcLengthFilter(RSProbes As Recordset, RSPS As Recordset)
'-------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for Length filter calculation.
'Arguments
'   RSProbes: The probes recordset (calculate Length Filter for all records).
'   RSPS: The parameter recordset (set parameters used in current record).
'-------------------------------------------------------------------------
On Error GoTo E

Dim PSName$                     'name of current probeset
Dim NumProbes&                  'number of probes in the recordset
Dim Length&()                   'Length column from database
Dim LengthFilter() As Boolean   'Length Filter column to database

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly

'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating Length Filter for ProbeSet " & PSName, 0,
NumProbes
```

```
'Create space for database fields.
ReDim Length(0 To NumProbes - 1)
ReDim LengthFilter(0 To NumProbes - 1)

'Get the Lengths.
GetField RSProbes, "Length", Length

'Calculate Length Filter.
CalcLengthFilter Length, LengthFilterPars, LengthFilter

'Update the results.
Progress.ShowProgress "Updating Length Filter in Database for ProbeSet " &
PSName, 0, NumProbes
PutField RSProbes, "Length Filter", LengthFilter

UpdateOnly:

'Store parameters in the database, and update column visibility.
LengthFilterPars.StoreDB RSPS
PSColumns("Length Filter").IsFilter = LengthFilterPars.AppImmediate

Exit Sub
E: Debug.Print "Error in PSCalcLengthFilter"
Err.Raise Err.Number, , Err.Description
End Sub

Private Sub PSCalcPosFilter(RSProbes As Recordset, RSPS As Recordset)
'-------------------------------------------------------------------------
'Function
'   Perform all database gets/puts, etc, for Pos filter calculation.
'Arguments
'   RSProbes: The probes recordset (calculate Pos Filter for all records).
'   RSPS: The parameter recordset (set parameters used in current record).
'-------------------------------------------------------------------------
On Error GoTo E

Dim PSName$                     'name of current probeset
Dim NumProbes&                  'number of probes in the recordset
Dim Pos&()                      'Pos column from database
Dim PosFilter() As Boolean      'Pos Filter column to database
Dim SeqLength&                  'Length of the originating sequence.

'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly

'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating Pos Filter for ProbeSet " & PSName, 0,
NumProbes

'Create space for database fields.
ReDim Pos(0 To NumProbes - 1)
ReDim PosFilter(0 To NumProbes - 1)

'Get the Positions.
GetField RSProbes, "Position", Pos
```



```
'Get the sequence length.
SeqLength = RSPS("CreatePS-SeqLength")

'Calculate Pos Filter.
CalcPosFilter Pos, SeqLength, PosFilterPars, PosFilter

'Update the results.
Progress.ShowProgress "Updating Pos Filter in Database for ProbeSet " & PSName,
0, NumProbes
PutField RSProbes, "Pos Filter", PosFilter

UpdateOnly:

'Store parameters in the database, and update column visibility.
PosFilterPars.StoreDB RSPS
PSColumns("Pos Filter").IsFilter = PosFilterPars.AppImmediate

Exit Sub
E: Debug.Print "Error in PSCalcPosFilter"
Err.Raise Err.Number, , Err.Description
End Sub
```

```
Attribute VB_Name = "Filters"
Option Explicit

Public Sub CalcTMFilter(TM#(), TMFPars As cTMFilterPars, Filter() As Boolean)
'-------------------------------------------------------------------------
'Function
'   Decide which probes pass the TM filter.
'Arguments
'   TM: An array of TMs
'   TMFPars: An instance of the parameter class cTMFilterPars.
'   Filter: The return array of filter output values.
'-------------------------------------------------------------------------

'The TM filter can be implemented directly by the generic filter.
CalcGenericFilter TM, TMFPars, Filter
End Sub


Public Sub CalcdGDFilter(dGD#(), dGDFPars As cdGDFilterPars, Filter() As
Boolean)
'-------------------------------------------------------------------------
'Function
'   Decide which probes pass the dGD filter.
'Arguments
'   dGD: An array of dGDs
'   dGDFPars: An instance of the parameter class cdGDFilterPars.
'   Filter: The return array of filter output values.
'-------------------------------------------------------------------------

'The dGD filter can be implemented directly by the generic filter.
CalcGenericFilter dGD, dGDFPars, Filter
End Sub
```

```
Public Sub CalcGenericFilter(Values, FilterPars As Object, Filter() As Boolean)
'-------------------------------------------------------------------------------
'Function
'   Decide which values pass the filter.
'Arguments
'   Values: An array of values to be filtered against.
'   FilterPars: The filter parameters
'   Filter: The returned filter settings.
'Notes
'   This routine is used to implement the common filter methods.  FilterPars is
'   declared as an object rather than a specific class, and thus needs to have
'   only the members accessed on the execution path.
'   FilterPars must have data member Method.
'   If method is None, no other members are required.
'   If method is Min, then member Min is required.
'   If method is Max, then member Max is required.
'   If method is InRange, then members Max and Min are required.
'   If method is Distance, then members Target and Distance are required.
'   If method is NBest, then members Target and NBest are required.
'   If method is Percent, then members Target and Percent are required.
'History
'   31-Jul-97: PW
'-------------------------------------------------------------------------------

Dim Distance#()             'absolute difference between values and target
Dim Target#                 'target value
Dim MinValue#, MaxValue#    'min and max of value
Dim Index&()                'index array to track sort
Dim NumValues&              'number of values we are working with
Dim T&                      'index
Dim N&                      'number to set.

'Determine the number of probes we are calculating filter for.
NumValues = UBound(Values) + 1

'If method is "None", set all to true.
If FilterPars.Method = "None" Then
    For T = 0 To NumValues - 1
        Filter(T) = True
    Next T
    Exit Sub
End If

'If method is "Min", set all >= min to true.
If FilterPars.Method = "Min" Then
    For T = 0 To NumValues - 1
        Filter(T) = (Values(T) >= Val(FilterPars.Min))
    Next T
    Exit Sub
End If

'If method is "Max", set all <= max to true.
If FilterPars.Method = "Max" Then
    For T = 0 To NumValues - 1
        Filter(T) = (Values(T) <= Val(FilterPars.Max))
    Next T
    Exit Sub
End If
```



```
'If method is "InRange", set all >= min and <= max to true.
If FilterPars.Method = "InRange" Then
    For T = 0 To NumValues - 1
        Filter(T) = ((Values(T) >= Val(FilterPars.Min)) And (Values(T) <=
Val(FilterPars.Max)))
    Next T
    Exit Sub
End If

'For other methods, we have to do more work!

'Size the arrays.
ReDim Distance(NumValues - 1)
ReDim Index(NumValues - 1)

'Find the minimum and maximum values.
For T = 0 To NumValues - 1
    If MinValue > Values(T) Then MinValue = Values(T)
    If MaxValue < Values(T) Then MaxValue = Values(T)
Next T

'Choose the target value.
If (FilterPars.Method = "Distance") Or _
   (FilterPars.Method = "NBest") Or _
   (FilterPars.Method = "Percent") _
    Then Target = FilterPars.Target
If (FilterPars.Method = "NLowest") Or _
   (FilterPars.Method = "PercentLowest") _
    Then Target = MinValue
If (FilterPars.Method = "NHighest") Or _
   (FilterPars.Method = "PercentHighest") _
    Then Target = MaxValue

'Compute distances of Values from target; set up index and filter.
For T = 0 To NumValues - 1
    Distance(T) = Abs(Values(T) - Target)
    Index(T) = T
    Filter(T) = False
Next T

'If method is "Distance", set all elements within distance as true.
If FilterPars.Method = "Distance" Then
    For T = 0 To NumValues - 1
        Filter(T) = Distance(T) <= FilterPars.Distance
    Next T
    Exit Sub
End If

'Choose the number to set
If (FilterPars.Method = "NBest") Or _
   (FilterPars.Method = "NLowest") Or _
   (FilterPars.Method = "NHighest") _
    Then N = FilterPars.NBest
If (FilterPars.Method = "Percent") Or _
   (FilterPars.Method = "PercentLwest") Or _
   (FilterPars.Method = "PercentHighest") _
    Then N = NumValues * FilterPars.Percent / 100 - 1
```

```
    If N >= NumValues Then N = NumValues - 1

    'Sort on distance from target.
    QuickSort Distance, Index, 0, NumValues - 1

    'Set the best.
    For T = 0 To N - 1
        Filter(Index(T)) = True
    Next T

    End Sub


    Public Sub CalcRunFilter(Run&(), RunFPars As CRunFilterPars, Filter() As
    Boolean)
    '-------------------------------------------------------------------------
    'Function
    '   Decide which probes pass the Run filter.
    'Arguments
    '   Run: An array of Runs
    '   RunFPars: An instance of the parameter class cRunFilterPars.
    '   Filter: The return array of filter output values.
    '-------------------------------------------------------------------------
    Dim OldMin&

    'The special method "All" can be handled by using the method Min, with min=1.
    If RunFPars.Method = "All" Then
        OldMin = RunFPars.Min
        RunFPars.Min = 1
        RunFPars.Method = "Min"
        CalcGenericFilter Run, RunFPars, Filter
        RunFPars.Method = "All"
        RunFPars.Min = OldMin
        Exit Sub
    End If

    'Otherwise, filters are generic.
    CalcGenericFilter Run, RunFPars, Filter

    End Sub


    Public Sub CalcdGMFilter(dGM#(), dGMFPars As cdGMFilterPars, Filter() As
    Boolean)
    '-------------------------------------------------------------------------
    'Function
    '   Decide which probes pass the dGM filter.
    'Arguments
    '   dGM: An array of dGMs
    '   dGMFPars: An instance of the parameter class cdGMFilterPars.
    '   Filter: The return array of filter output values.
    '-------------------------------------------------------------------------

    CalcGenericFilter dGM, dGMFPars, Filter
    End Sub
```



```
    Public Sub CalcClampFilter(Clamp#(), ClampFPars As cClampFilterPars, Filter() As
    Boolean)
    '-------------------------------------------------------------------------
    'Function
    '   Decide which probes pass the Clamp Filter.
    'Arguments
    '   Clamp: An array of Clamps
    '   ClampFPars: An instance of the parameter class cClampFilterPars.
    '   Filter: The return array of filter output values.
    'Notes
    'History
    '   31-Jul-97: PW
    '-------------------------------------------------------------------------

    CalcGenericFilter Clamp, ClampFPars, Filter
    End Sub

    Public Sub CalcGCFilter(GC#(), GCFPars As cGCFilterPars, Filter() As Boolean)
    '-------------------------------------------------------------------------
    'Function
    '   Decide which probes pass the GC filter.
    'Arguments
    '   GC: An array of GCs
    '   GCFPars: An instance of the parameter class cGCFilterPars.
    '   Filter: The return array of filter output values.
    '-------------------------------------------------------------------------

    CalcGenericFilter GC, GCFPars, Filter
    End Sub

    Public Sub CalcdGHFilter(dGH#(), dGHFPars As cdGHFilterPars, Filter() As
    Boolean)
    '-------------------------------------------------------------------------
    'Function
    '   Decide which probes pass the dGH filter.
    'Arguments
    '   dGH: An array of dGHs
    '   dGHFPars: An instance of the parameter class cdGHFilterPars.
    '   Filter: The return array of filter output values.
    '-------------------------------------------------------------------------

    CalcGenericFilter dGH, dGHFPars, Filter
    End Sub


    Public Sub CalcHomoFilter(Homology&(), HFPars As cHomoFilterPars, Filter() As
    Boolean)
    '-------------------------------------------------------------------------
    'Function
    '   Decide which probes pass the homology filter.
    'Arguments
    '   Homology: An array of homologiess
    '   HFPars: An instance of the parameter class cHomoFilterPars.
    '   Filter: The return array of filter output values.
    '-------------------------------------------------------------------------

    CalcGenericFilter Homology, HFPars, Filter
```

```
End Sub


Public Sub CalcPosFilter(Pos&(), SeqLength&, PFPars As cPosFilterPars, Filter()
As Boolean)
'-------------------------------------------------------------------------------
'Function
'   Decide which probes pass the position filter.
'Arguments
'   Pos: An array of positions.
'   SeqLength: The length of the sequence from which the probeset was derived.
'   PFPars: An instance of the parameter class cPosFilterPars.
'   Filter: The return array of filter output values.
'-------------------------------------------------------------------------------

'The target for this filter should be the specified end.
If PFPars.WhichEnd = "5-prime" Then
    PFPars.Target = 1
Else
    PFPars.Target = SeqLength
End If
CalcGenericFilter Pos, PFPars, Filter
End Sub

Public Sub CalcLengthFilter(Length&(), LFPars As cLengthFilterPars, Filter() As
Boolean)
'-------------------------------------------------------------------------------
'Function
'   Decide which probes pass the length filter.
'Arguments
'   Length: An array of lengths.
'   LFPars: An instance of the parameter class cLengthFilterPars.
'   Filter: The return array of filter output values.
'-------------------------------------------------------------------------------
CalcGenericFilter Length, LFPars, Filter
End Sub
```

```
Attribute VB_Name = "GenBank"
Option Explicit

Private Const GBTerminator$ = "//"      'record terminator in Genbank files
Private Const MaxGBFileLength& = 100000 'maximum allowed GenBank file length

Public Sub EntrezLoadDB(Accession$, RS As Recordset, KeepFile As Boolean)
'-------------------------------------------------------------------------------
'Function
'   Grab a sequence using the Entrez CGI.
'Arguments
'   Accession: The accession of the required sequence.
'   RS: The recordset into which this sequence should be loaded.
'   KeepFile: Controls whether the downloaded file is retained or removed.
'-------------------------------------------------------------------------------
On Error GoTo E

Dim GBFileName$                 'Genbank sequence file for data.
Dim EntrezData$, EntrezData2$   'GB Entries.
Dim i&
```



```
'Grab the data.
EntrezData = frmEntrezGrab.Entrez.GetGenBankData(Accession, "")

'Create CRLFs out of LFs.
For i = 1 To Len(EntrezData)
    If (Mid$(EntrezData, i, 1) = vbLf) Then EntrezData2 = EntrezData2 + vbCr
    EntrezData2 = EntrezData2 + Mid$(EntrezData, i, 1)
Next i

'Write the aquired data out to a file.
GBFileName = frmMain.cdgSeqs.InitDir & "\" & Accession & ".gb"
Open GBFileName For Output As #1
Print #1, EntrezData2
Close #1

'Load the database.
SeqLoadDB ReadGBRecord(GBFileName), RS

'Remove the file.
If Not KeepFile Then Kill GBFileName

Exit Sub
E: Debug.Print "Error in EntrezLoadDB"
Err.Raise Err.Number, , Err.Description
End Sub

Public Sub SeqLoadDB(SeqRC As SeqRecord, RS As Recordset)
'-------------------------------------------------------------------------------
'Function
'   Create a new record in the Sequence table, populate from SeqRC
'Arguments
'   SeqRC: A sequence structure, as read from file, etc.
'   RS: The recordset into which the sequence should be loaded.
'Errors:
'   Attempting to create a new sequence record whose Accession matches a
'   previously inserted sequence causes a DB error.
'-------------------------------------------------------------------------------
On Error GoTo E

BeginTrans
    AllowdbgClick = False
    RS.AddNew
    RS.Fields("Header") = SeqRC.Header
    RS.Fields("Accession") = SeqRC.Accession
    RS.Fields("Locus") = SeqRC.Locus
    RS.Fields("Length") = SeqRC.Length
    RS.Fields("Sequence") = SeqRC.Sequence
    RS.Fields("Selected") = True
    RS.Update
    RS.Bookmark = RS.LastModified
CommitTrans
DoEvents
AllowdbgClick = True
Exit Sub
E: Debug.Print "Error in SeqLoadDB"
Rollback
DoEvents
```

```
AllowdbgClick = True
Err.Raise Err.Number, , Err.Description
End Sub

Public Function ReadGBRecord(FileName$) As SeqRecord
'--------------------------------------------------------------------------
'Function
'   Read a GenBank record from a file into a SeqRecord.
'Arguments
'   FileName: The filename of the GB record.
'Returns
'   The GenBank record as a SeqRecord.
'Errors:
'   Returned to caller.
'   1. Check file is less than MaxGBFileLength
'   2. Check that all required fields are present.
'--------------------------------------------------------------------------
On Error GoTo E

Dim GBFile&             'GenBank file
Dim FileLength&         'file length (chars)
Dim LineLength&         'length of current line (chars)

Dim GBText$             'entire record
Dim GBItem$             'one line
Dim KeyField$           'item keyfield
Dim foo$                'bit bucket

Dim GB As SeqRecord     'the returned record

'open the file
GBFile = FreeFile
Open FileName For Binary As #GBFile

'read in the file as one long string
FileLength = FileLen(FileName)
If FileLength > MaxGBFileLength Then Err.Raise ppErrGBFileLength
GBText = Input(FileLength, #GBFile)

'check for required fields
If InStr(GBText, "LOCUS") = 0 Then Err.Raise ppErrGBFileFormat, , "No LOCUS
field found in GB file."
If InStr(GBText, "ACCESSION") = 0 Then Err.Raise ppErrGBFileFormat, , "No
ACCESSION field found in GB file."
If InStr(GBText, GBTerminator) = 0 Then Err.Raise ppErrGBFileFormat, , "No
terminator (//) found in GB file."

'process items
Do While True

    'extract an item
    LineLength = InStr(GBText, vbCrLf) - 1
    GBItem = Left(GBText, LineLength)
    GB.Header = GB.Header & GBItem & vbCrLf
    GBText = Right(GBText, FileLength - LineLength - 2)
    FileLength = FileLength - LineLength - 2

    If Left(GBItem, 2) = GBTerminator Then
```



```
        GoTo breakwhile1
    End If

    'extract keyfield, and process item
    KeyField = StrField(GBItem, LineLength, 10)
    'if keyfield is not numeric -> header
    If IsNumeric(KeyField) = False Then
        'if keyfield occupies first column -> keyword
        If Left(KeyField, 1) <> " " Then
            'process keywords
            Select Case KeyField
            Case "LOCUS"
                foo = StrField(GBItem, LineLength, 2)
                GB.Locus = StrField(GBItem, LineLength, 10)
                GB.Length = StrField(GBItem, LineLength, 7)
            Case "ACCESSION"
                foo = StrField(GBItem, LineLength, 2)
                GB.Accession = StrField(GBItem, LineLength, 6)
            End Select
        'if keyfield has only two blanks -> subkeyword
        ElseIf Left(KeyField, 3) <> "   " Then
        'if keyfield has only 4 blanks -> feature code
        ElseIf Left(KeyField, 5) <> "     " Then
        'it must be a continuation line
        Else
        End If
    Else
        'sequence has started
        Do While LineLength > 10
            GB.Sequence = GB.Sequence & Left(GBItem, 10)
            GBItem = Right(GBItem, LineLength - 11)
            LineLength = LineLength - 11
        Loop
        If LineLength > 0 Then
            GB.Sequence = GB.Sequence & GBItem
        End If
    End If
Loop
breakwhile1:
Close GBFile

'Strip any whitespace.
GB.Sequence = PackSequence(GB.Sequence)

'Check sequence length.
If GB.Length <> Len(GB.Sequence) Then
    MsgBox "Sequence length differs from header specification."
    GB.Length = Len(GB.Sequence)
    GB.Accession = GB.Accession & "*"
End If

ReadGBRecord = GB
Exit Function
E: Debug.Print "Error in ReadGBRecord"
Err.Raise Err.Number, , Err.Description
End Function
```

```
Public Function ReadFASTARecord(FASTAFile&) As SeqRecord
'--------------------------------------------------------------------------------
'Function
'   Read a FASTA record from a file into a SeqRecord.
'Arguments
'   FASTA: The file descriptor for the FASTA file.
'Returns
'   The FASTA record as a SeqRecord.
'Errors:
'   Returned to caller.
'Notes:
'   The FASTA file must already be opened for input, and should be positioned
'   at the start of a FASTA header.  It will read an entire sequence, leaving
'   the file positioned on the subsequent header.
'--------------------------------------------------------------------------------
On Error GoTo E

Dim LineText$           'one line of the file
Dim SR As SeqRecord     'the returned record

'Read the header line, check it is legal.
Line Input #FASTAFile, LineText
If Left(LineText, 1) <> ">" Then Err.Raise ppErrFASTAFormat, , "Header line does
not begin w/ >."

'Extract the length.
LineText = Right(LineText, Len(LineText) - InStr(LineText, "len=") - 3)
If InStr(LineText, "/") <> 0 Then LineText = Left(LineText, InStr(LineText, "/")
- 1)
SR.Length = CLng(LineText)

'Read sequence lines till we are done.
Do While (Len(SR.Sequence) <> SR.Length)
    Line Input #FASTAFile, LineText
    SR.Sequence = SR.Sequence & LineText
Loop

ReadFASTARecord = SR

Exit Function
E: Debug.Print "Error in ReadFASTARecord"
Err.Raise Err.Number, , Err.Description
End Function

Public Function ReadFASTAHeader(FASTAFile&) As SeqRecord
'--------------------------------------------------------------------------------
'Function
'   Read a FASTA header from a file into a SeqRecord.
'Arguments
'   FASTA: The file descriptor for the FASTA header file.
'Returns
'   The FASTA record as a SeqRecord.
'Errors:
'   Returned to caller.
'Notes:
'   The FASTA header file must already be opened for input, and should be
positioned
'   at the start of a FASTA header.
```



```
'--------------------------------------------------------------------------------
On Error GoTo E

Dim LineText$           'one line of the file
Dim LenText$            'line processed to find length
Dim AccText$            'line processed to find accession
Dim SR As SeqRecord     'the returned record

'Read the header line, check it is legal.
Line Input #FASTAFile, LineText
If Left(LineText, 1) <> ">" Then Err.Raise ppErrFASTAFormat, , "Header line does
not begin w/ >."

'Extract the length.
LenText = Right(LineText, Len(LineText) - InStr(LineText, "len=") - 3)
If InStr(LenText, "/") <> 0 Then LenText = Left(LenText, InStr(LenText, "/") -
1)
SR.Length = CLng(LenText)

'Extract the accession.
AccText = Right(LineText, Len(LineText) - InStr(LineText, "gb=") - 2)
If InStr(AccText, "/") <> 0 Then AccText = Left(AccText, InStr(AccText, "/") -
1)
SR.Accession = LTrim(RTrim(AccText))

ReadFASTAHeader = SR

Exit Function
E: Debug.Print "Error in ReadFASTARecord"
Err.Raise Err.Number, , Err.Description
End Function


Public Function GBCookSeq(ByVal RawSeq$) As String
'Convert raw sequence (no spaces, etc) into more
'palatable form, with spaces every 10 nucleotides
'and newlines every 60 nucleotides.

Dim Bases&          'count the bases
Dim LineLength&     'remaining linelength

LineLength = Len(RawSeq)
Bases = 1
Do While LineLength > 60
    GBCookSeq = GBCookSeq &
        Format(Format(Bases, "#####"), "@@@@@") & " "
    Bases = Bases + 60
    Dim i&
    For i = 1 To 6
        GBCookSeq = GBCookSeq & StrField(RawSeq, LineLength, 10) & " "
    Next i
    GBCookSeq = GBCookSeq & vbCrLf
Loop
If Len(RawSeq) <> 0 Then
    GBCookSeq = GBCookSeq &
        Format(Format(Bases, "#####"), "@@@@@") & " "
    Do While Len(RawSeq) > 10
        GBCookSeq = GBCookSeq & StrField(RawSeq, LineLength, 10) & " "
```

```
    Loop
    GBCookSeq = GBCookSeq & RawSeq
End If
End Function

Public Function RTFGBCookSeq(yVal RawSeq$) As String
'Convert raw sequence (no spaces, etc) into more
'palatable form, with spaces every 10 nucleotides
'and newlines every 60 nucleotides.

Dim Bases&          'count the bases
Dim LineLength&     'remaining linelength

LineLength = Len(RawSeq)
Bases = 1
Do While LineLength > 60
    RTFGBCookSeq = RTFGBCookSeq &
        Format(Format(Bases, "#####"), "@@@@@") & " "
    Bases = Bases + 60
    Dim i&
    For i = 1 To 6
        RTFGBCookSeq = RTFGBCookSeq & StrField(RawSeq, LineLength, 10) & " "
    Next i
    RTFGBCookSeq = RTFGBCookSeq & "\par"
Loop
If Len(RawSeq) <> 0 Then
    RTFGBCookSeq = RTFGBCookSeq &
        Format(Format(Bases, "#####"), "@@@@@") & " "
    Do While Len(RawSeq) > 10
        RTFGBCookSeq = RTFGBCookSeq & StrField(RawSeq, LineLength, 10) & " "
    Loop
    RTFGBCookSeq = RTFGBCookSeq & RawSeq
End If
End Function
```

```
Attribute VB_Name = "SQL"
Option Explicit

Public Function BuildPOnePSQuery(PSName)
'-------------------------------------------------------------
'Function
'   Build an SQL Query to select Probes from the Probes table
'   that pass all filters, and come from the named ProbeSet.
'Arguments
'   Accession: The accession to search on.
'   PSName: The ProbeSet name to search on.
'Notes
'   2. All fields corresponding to a column (that is, all fields
'      named in the Columns table) are returned, to match the layout
'      of the Probes grid.  The visibility of various columns is
'      controlled by the column Visible properties.
'   3. The currently set filters are used as a further selection
'      on the probes returned.
'-------------------------------------------------------------
Dim SelectC$, FromC$, FilterC$, WhereC$, OrderByC$
Dim C&, F&
```



```
'Set up the select clause -- return all columns.
SelectC = "SELECT * "
'For C = 1 To PSColumns.Count
'    If C <> 1 Then SelectC = SelectC & ", "
'    SelectC = SelectC & "[" & PSColumns(C).Name & "] "
'Next C

'Set up the the from clause -- always the Probes table.
FromC = "FROM Probes "

'Set up the where clause
For F = 1 To PSColumns.Count
    If (PSColumns(F).IsFilter = True) Then
        If FilterC = "" Then
            FilterC = "([" & PSColumns(F).Name & "] = True)"
        Else
            FilterC = FilterC & " AND ([" & PSColumns(F).Name & "] = True)"
        End If
    End If
Next F
WhereC = "WHERE ( (PSName = '" & PSName & "')"
If PSColumns("User Filter").IsFilter Then
    If FilterC <> "" Then
        WhereC = WhereC & " AND ( ([User Filter] = 2) OR ( ([User Filter] = 1)
AND " & FilterC & ") ) ) "
    Else
        WhereC = WhereC & " AND ([User Filter] <> 0)) ) "
    End If
Else
    If FilterC <> "" Then
        WhereC = WhereC & " AND ( " & FilterC & " ) ) "
    Else
        WhereC = WhereC & " ) "
    End If
End If

'Put it all together.
BuildPOnePSQuery = SelectC & FromC & WhereC & ";"


End Function

Public Function BuildProbesQuery()
'-------------------------------------------------------------
'Function
'   Build an SQL Query to select Probes from the Probes table.
'Arguments
'   Accession: The accession to search on.
'   PSName: The ProbeSet name to search on.
'Notes
'   1. Accession or PSName = <none> is allowed, and results
'      in an empty recordset.
'   2. All fields corresponding to a column (that is, all fields
'      named in the Columns table) are returned, to match the layout
'      of the Probes grid.  The visibility of various columns is
'      controlled by the column Visible properties.
'   3. The currently set filters are used as a further selection
'      on the probes returned.
```

```
'-------------------------------------------------------------
Dim SelectC$, FromC$, FilterC$, WhereC$, OrderByC$
Dim C&, F&
Dim Col As cColumn

'Set up the select clause -- return all columns.
SelectC = "SELECT * "
'For C = 1 To PSColumns.Count
'    If C <> 1 Then SelectC = SelectC & ", "
'    SelectC = SelectC & "Probes.[" & PSColumns(C).Name & "] "
'Next C

'Set up the the from clause -- from the SelectProbes query.
FromC = "FROM SelectProbes "

'Set up the where clause -- filter the rows.
'For F = 1 To PSColumns.count
For Each Col In PSColumns
    If (Col.IsFilter = True) Then
        If FilterC = "" Then
            FilterC = "([" & Col.Name & "] = True)"
        Else
            FilterC = FilterC & " AND ([" & Col.Name & "] = True)"
        End If
    End If
Next Col
WhereC = "WHERE ( (Selected = True)"
If PSColumns("User Filter").IsFilter Then
    If FilterC <> "" Then
        WhereC = WhereC & " AND ( ([User Filter] = 2) OR ( ([User Filter] = 1)
AND " & FilterC & ") ) ) "
    Else
        WhereC = WhereC & " AND ([User Filter] <> 0)) ) "
    End If
Else
    If FilterC <> "" Then
        WhereC = WhereC & " AND ( " & FilterC & " ) ) "
    Else
        WhereC = WhereC & " ) "
    End If
End If

'Set up the orderby clause.
'Default to sorting by position, then check columns.
OrderByC = "Position "
For Each Col In PSColumns
    If (Col.IsSort = "Ascending") Then OrderByC = "[" & Col.Name & "] ASC "
    If (Col.IsSort = "Descending") Then OrderByC = "[" & Col.Name & "] DESC "
Next Col
OrderByC = "ORDER BY Probes.Accession, Probes.PSName, " & OrderByC

'Put it all together.
BuildProbesQuery = SelectC & FromC & WhereC & OrderByC & ";"

End Function
```

```
Attribute VB_Name = "Stats"
```



```
Option Explicit
Public Type StatRecord
    Count As Long
    Min As Double
    Ten As Double
    Median As Double
    Ninety As Double
    Max As Double
    Range As Double
    Average As Double
    Std As Double
End Type


Public Function Statistics(Vector#()) As StatRecord
'-------------------------------------------------------------------------
'Function
'   Calculate all statistics of a vector.
'-------------------------------------------------------------------------
Dim L&, U&
Dim Min#, Max#, Total#, Total2#
Dim Index&()
Dim i&

'Get array bounds.
L = LBound(Vector)
U = UBound(Vector)

'Set the count.
Statistics.Count = UBound(Vector) - LBound(Vector) + 1
ReDim Index(L To U)

'Take a pass through to get min, max, total, total^2, index.
Min = Vector(L)
Max = Vector(L)
For i = L To U
    Index(i) = i
    Total = Total + Vector(i)
    Total2 = Total2 + Vector(i) * Vector(i)
    If Vector(i) > Max Then Max = Vector(i)
    If Vector(i) < Min Then Min = Vector(i)
Next i

'Set min, max, range, average, std.
Statistics.Min = Min
Statistics.Max = Max
Statistics.Range = Max - Min
Statistics.Average = Total / Statistics.Count
Statistics.Std = Sqr((Total2 / Statistics.Count) - Statistics.Average *
Statistics.Average)

'Sort.
QuickSort Vector, Index, L, U

'Percentiles.
Statistics.Ten = Vector(L + (Statistics.Count - 1) * 0.1)
Statistics.Median = Vector(L + (Statistics.Count - 1) * 0.5)
Statistics.Ninety = Vector(L + (Statistics.Count - 1) * 0.9)
```

```
End Function
```

```
Attribute VB_Name = "Thermo"
Option Explicit

'There is no good reason to have two sets of thermodynamic parameters,
'other than history...

'Parameters used by TM calculations.
Public Const RGas# = 1.987                  'Gas constant, cal/mol/deg K.
Private DNA_DuplexH#(0 To 3, 0 To 3)        'DNA/DNA nearest neighbor
enthalpies.
Private DNA_DuplexS#(0 To 3, 0 To 3)        'DNA/RNA nearest neighbor entropies.
Private DNA_InitGCS#, DNA_InitATS#          'DNA/DNA initiation entropies.
Private DNA_SelfS#                          'DNA/DNA self-symmetry entropies.
Private DNA_EndTAH#                         'DNA/DNA TA end enthalpy.
Private DR_DuplexH#(0 To 3, 0 To 3)         'DNA/RNA nearest neighbor
enthalpies.
Private DR_DuplexS#(0 To 3, 0 To 3)         'DNA/RNA nearest neighbor entropies.
Private DR_InitH#, DR_InitS#                'DNA/RNA initiation
enthaply/entropy.

'Parameters used by hairpin calculations.
'Zuker provides H and G @ 37.  So on load, calculate S, then recalculate G as
needed.
Private Const NumZukerLoops& = 30               'Number of special case loops.
Private ZLoopLengths&(0 To NumZukerLoops - 1)   'The special case loop lengths.
Private Const DNA_ZMiscLoop# = 1.079
Private Const RNA_ZMiscLoop# = 1.079            'Constants for large-loop
entropies.
Private Const ZMinLoop& = 4                     'Minumum allowed loop.
Private Const NumTetraLoops& = 8                'Number of special tetraloops.
Private ZTetraLoops&(0 To NumTetraLoops - 1)    'Indices of tetraloops.

'DNA free energy.
Private DNAHP_StackG#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private DNAHP_TStackG#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private DNAHP_DangleG#(0 To 1, 0 To 3, 0 To 3, 0 To 3)
Private DNAHP_LoopG#(0 To 2, 0 To NumZukerLoops - 1)
Private DNAHP_TLoopG#(0 To NumTetraLoops - 1)

'DNA enthalpy.
Private DNAHP_StackH#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private DNAHP_TStackH#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private DNAHP_DangleH#(0 To 1, 0 To 3, 0 To 3, 0 To 3)
Private DNAHP_LoopH#(0 To 2, 0 To NumZukerLoops - 1)
Private DNAHP_TLoopH#(0 To NumTetraLoops - 1)

'DNA entropy.
Private DNAHP_StackS#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private DNAHP_TStackS#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private DNAHP_DangleS#(0 To 1, 0 To 3, 0 To 3, 0 To 3)
Private DNAHP_LoopS#(0 To 2, 0 To NumZukerLoops - 1)
Private DNAHP_TLoopS#(0 To NumTetraLoops - 1)

'RNA free energy.
```



```
Private RNAHP_StackG#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private RNAHP_TStackG#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private RNAHP_DangleG#(0 To 1, 0 To 3, 0 To 3, 0 To 3)
Private RNAHP_LoopG#(0 To 2, 0 To NumZukerLoops - 1)
Private RNAHP_TLoopG#(0 To NumTetraLoops - 1)

'RNA enthalpy.
Private RNAHP_StackH#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private RNAHP_TStackH#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private RNAHP_DangleH#(0 To 1, 0 To 3, 0 To 3, 0 To 3)
Private RNAHP_LoopH#(0 To 2, 0 To NumZukerLoops - 1)
Private RNAHP_TLoopH#(0 To NumTetraLoops - 1)

'RNA entropy.
Private RNAHP_StackS#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private RNAHP_TStackS#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private RNAHP_DangleS#(0 To 1, 0 To 3, 0 To 3, 0 To 3)
Private RNAHP_LoopS#(0 To 2, 0 To NumZukerLoops - 1)
Private RNAHP_TLoopS#(0 To NumTetraLoops - 1)

Private Sub CalcSFromGH(Dims&, G#(), H#(), ByVal T#, S#())
'------------------------------------------------------------------------------
'Function
'   Calculate S given G, H, and T, for all elements of array S.
'Arguments
'   Dims: The number of dimensions of G, H, and S.
'   G: The given free energies.
'   H: The given enthalpies.
'   T: The temperature at which free energy was calculated.
'   S: The returned entropies.
'Notes
'   1. The reason for this functions existence is that Zuker provides
'   G at 37, and H.  S is needed to change temperatures.
'------------------------------------------------------------------------------
Dim I&, J&, K&, L&
T = T + 273.15
Select Case Dims
Case 1
    For I = LBound(G) To UBound(G)
        S(I) = (G(I) - H(I)) / T
    Next I
Case 2
    For I = LBound(G, 1) To UBound(G, 1)
        For J = LBound(G, 2) To UBound(G, 2)
            S(I, J) = (G(I, J) - H(I, J)) / T
        Next J
    Next I
Case 4
    For I = LBound(G, 1) To UBound(G, 1)
        For J = LBound(G, 2) To UBound(G, 2)
            For K = LBound(G, 3) To UBound(G, 3)
                For L = LBound(G, 4) To UBound(G, 4)
                    S(I, J, K, L) = (G(I, J, K, L) - H(I, J, K, L)) / T
                Next L
            Next K
        Next J
    Next I
End Select
```



```
End Sub

Private Sub CalcGFromHS(Dims&, H#(), S#(), ByVal T#, G#())
'-------------------------------------------------------------------------
'Function
'   Calculate G given H, S, and T, for all elements of array G.
'Arguments
'   Dims: The number of dimensions of G, H, and S.
'   H: The given enthalpies.
'   S: The given entropies.
'   T: The temperature at which free energy is required.
'   S: The returned free energies.
'-------------------------------------------------------------------------
Dim I&, J&, K&, L&
T = T + 273.15
Select Case Dims
Case 1
    For I = LBound(G) To UBound(G)
        G(I) = 0.1 * CDbl(CInt(10# * (H(I) + S(I) * T)))
    Next I
Case 2
    For I = LBound(G, 1) To UBound(G, 1)
        For J = LBound(G, 2) To UBound(G, 2)
            G(I, J) = 0.1 * CDbl(CInt(10# * (H(I, J) + S(I, J) * T)))
        Next J
    Next I
Case 4
    For I = LBound(G, 1) To UBound(G, 1)
        For J = LBound(G, 2) To UBound(G, 2)
            For K = LBound(G, 3) To UBound(G, 3)
                For L = LBound(G, 4) To UBound(G, 4)
                    G(I, J, K, L) = 0.1 * CDbl(CInt(10# * (H(I, J, K, L) + S(I,
J, K, L) * T)))
                Next L
            Next K
        Next J
    Next I
End Select
End Sub

Private Function DNA_BestHairpin#(Seq%())
'-------------------------------------------------------------------------
'Function
'   Compute the most stable hairpin for the given sequence.
'Arguments
'   Seq: The numerical representation of the sequence.
'-------------------------------------------------------------------------
Dim ThisHairpin#
Dim NumBases&
Dim FP&, TP&

DNA_BestHairpin = 1000#
NumBases = UBound(Seq) - LBound(Seq) + 1

For FP = 0 To NumBases - ZMinLoop - 2
    For TP = FP + ZMinLoop + 1 To NumBases - 1
        ThisHairpin = DNA_Hairpin(Seq, FP, TP)
        If ThisHairpin < DNA_BestHairpin Then DNA_BestHairpin = ThisHairpin
```



```
    Next TP
Next FP

End Function

Private Function RNA_BestHairpin#(Seq%())
'-------------------------------------------------------------------------
'Function
'   Compute the most stable hairpin for the given sequence.
'Arguments
'   Seq: The numerical representation of the sequence.
'-------------------------------------------------------------------------
Dim ThisHairpin#
Dim NumBases&
Dim FP&, TP&

RNA_BestHairpin = 1000#
NumBases = UBound(Seq) - LBound(Seq) + 1

For FP = 0 To NumBases - ZMinLoop - 2
    For TP = FP + ZMinLoop + 1 To NumBases - 1
        ThisHairpin = RNA_Hairpin(Seq, FP, TP)
        If ThisHairpin < RNA_BestHairpin Then RNA_BestHairpin = ThisHairpin
    Next TP
Next FP

End Function


Private Function RNA_Hairpin#(Seq%(), ByVal FP&, ByVal TP&)
'-------------------------------------------------------------------------
'Function
'   Calculate the free energy of a RNA hairpin
'Arguments
'   Seq: The sequence, in numeric representation.
'   FP: The index of the 5'-end base that closes the loop.
'   TP: The index of the 3' end base that closes the loop.
'-------------------------------------------------------------------------
Dim Energies#()
Dim NumBases, StemLength, S&

'Check whether this pair can close the loop.
RNA_Hairpin = 1000#
Select Case Seq(FP)
Case 0
    If Seq(TP) <> 3 Then Exit Function
Case 1
    If Seq(TP) <> 2 Then Exit Function
Case 2
    If (Seq(TP) <> 1 And Seq(TP) <> 3) Then Exit Function
Case 3
    If (Seq(TP) <> 0 And Seq(TP) <> 2) Then Exit Function
End Select

'Calculate sequence size.
NumBases = UBound(Seq) - LBound(Seq) + 1

'Setup to store all possible energies.
```

```
ReDim Energies(UBound(Seq) - LBound(Seq) + 1)

'Start the energy calculations with the loop.
Energies(0) = RNAHP_LoopG(2, TP - FP - 2)

'Add the stacking interaction of the first mismatch in the loop.
Energies(0) = Energies(0) + RNAHP_TStackG(Seq(FP), Seq(FP + 1), Seq(TP), Seq(TP
- 1))

'Loop over stem members
S = 0
Do While True
    FP = FP - 1
    TP = TP + 1

    'Check that there are still bases to process.
    If (FP >= 0) And (TP < NumBases) Then

        'Check that we are still in the helix.
        Select Case Seq(FP)
        Case 0
            If Seq(TP) <> 3 Then Exit Do
        Case 1
            If Seq(TP) <> 2 Then Exit Do
        Case 2
            If (Seq(TP) <> 1 And Seq(TP) <> 3) Then Exit Do
        Case 3
            If (Seq(TP) <> 0 And Seq(TP) <> 2) Then Exit Do
        End Select

        'Add the next stacking term
        Energies(S + 1) = Energies(S) + RNAHP_StackG(Seq(FP), Seq(FP + 1),
Seq(TP), Seq(TP - 1))

        'Record the energy if the helix breaks here.
        Energies(S) = Energies(S) + RNAHP_DangleG(1, Seq(TP - 1), Seq(FP + 1),
Seq(FP)) + _
            RNAHP_DangleG(0, Seq(TP - 1), Seq(TP), Seq(FP + 1))

        S = S + 1
    Else
        Exit Do
    End If
Loop

'Add dangles, if they exist.
If (FP >= 0) Then Energies(S) = Energies(S) + RNAHP_DangleG(1, Seq(TP - 1),
Seq(FP + 1), Seq(FP))
If (TP < NumBases) Then Energies(S) = Energies(S) + RNAHP_DangleG(0, Seq(TP -
1), Seq(TP), Seq(FP + 1))

'Find the minimum energy.
RNA_Hairpin = 1000#
StemLength = S
For S = 0 To StemLength
    If Energies(S) < RNA_Hairpin Then RNA_Hairpin = Energies(S)
Next S
```



```
End Function

Private Function DNA_Hairpin#(Seq%(), ByVal FP&, ByVal TP&)
'-------------------------------------------------------------------------
'Function
'   Calculate the free energy of a DNA hairpin
'Arguments
'   Seq: The sequence, in numeric representation.
'   FP: The index of the 5'-end base that closes the loop.
'   TP: The index of the 3' end base that closes the loop.
'-------------------------------------------------------------------------
Dim Energies#()
Dim NumBases, StemLength, S&

'Check whether this pair can close the loop.
DNA_Hairpin = 1000#
Select Case Seq(FP)
Case 0
    If Seq(TP) <> 3 Then Exit Function
Case 1
    If Seq(TP) <> 2 Then Exit Function
Case 2
    If (Seq(TP) <> 1 And Seq(TP) <> 3) Then Exit Function
Case 3
    If (Seq(TP) <> 0 And Seq(TP) <> 2) Then Exit Function
End Select

'Calculate sequence size.
NumBases = UBound(Seq) - LBound(Seq) + 1

'Setup to store all possible energies.
ReDim Energies(UBound(Seq) - LBound(Seq) + 1)

'Start the energy calculations with the loop.
Energies(0) = DNAHP_LoopG(2, TP - FP - 2)

'Add the stacking interaction of the first mismatch in the loop.
Energies(0) = Energies(0) + DNAHP_TStackG(Seq(FP), Seq(FP + 1), Seq(TP), Seq(TP
- 1))

'Loop over stem members
S = 0
Do While True
    FP = FP - 1
    TP = TP + 1

    'Check that there are still bases to process.
    If (FP >= 0) And (TP < NumBases) Then

        'Check that we are still in the helix.
        Select Case Seq(FP)
        Case 0
            If Seq(TP) <> 3 Then Exit Do
        Case 1
            If Seq(TP) <> 2 Then Exit Do
        Case 2
            If (Seq(TP) <> 1 And Seq(TP) <> 3) Then Exit Do
```

```
        Case 3
            If (Seq(TP) <> 0 And Seq(TP) <> 2) Then Exit Do
        End Select

        'Add the next stacking term
        Energies(S + 1) = Energies(S) + DNAHP_StackG(Seq(FP), Seq(FP + 1),
Seq(TP), Seq(TP - 1))

        'Record the energy if the helix breaks here.
        Energies(S) = Energies(S) + DNAHP_DangleG(1, Seq(TP - 1), Seq(FP + 1),
Seq(FP)) + _
            DNAHP_DangleG(0, Seq(TP - 1), Seq(TP), Seq(FP + 1))

        S = S + 1
    Else
        Exit Do
    End If
Loop

'Add dangles, if they exist.
If (FP >= 0) Then Energies(S) = Energies(S) + DNAHP_DangleG(1, Seq(TP - 1),
Seq(FP + 1), Seq(FP))
If (TP < NumBases) Then Energies(S) = Energies(S) + DNAHP_DangleG(0, Seq(TP -
1), Seq(TP), Seq(FP + 1))

'Find the minimum energy.
DNA_Hairpin = 1000#
StemLength = S
For S = 0 To StemLength
    If Energies(S) < DNA_Hairpin Then DNA_Hairpin = Energies(S)
Next S

End Function


Public Sub InitThermoPars()
'--------------------------------------------------------------------------
'Function:
'   Initialize all the fixed parameters used for Thermo calculations.
'Notes:
'   1. These parameters are essentially constants, but are placed in
'   arrays for ease-of-use.  Thus this routine must be called by
'   Main to initialize everything.
'   2. The nearest-neighbor parameters are accessed by indexing by
'   the first then second base, with A->0, C->1, G->2, TU->3
'   3. The hairpin parameters are loaded from files by this routine,
'   and the entropy matrices filled in.  After this operation, the
'   free energy matrices may be over-written at any time.
'--------------------------------------------------------------------------

Dim FileName$       'files opened for Zuker parameters.
Dim foo&()          'bitbucket.

'Initialize the DNA Duplex enthalpy and entropy.
'These parameters are from SantaLucia et al. Biochemistry, v. 35, pp 3555.
'as found by PkW.  DuplexH is in kcal/mol, DuplexS in cal/mol/deg K.
```



```
DNA_DuplexH(0, 0) = -8.4                'AA or TT
DNA_DuplexH(0, 1) = -8.6                'AC or GT
DNA_DuplexH(0, 2) = -6.1                'AG or CT
DNA_DuplexH(0, 3) = -6.5                'AT
DNA_DuplexH(1, 0) = -7.4                'CA or TG
DNA_DuplexH(1, 1) = -6.7                'CC or GG
DNA_DuplexH(1, 2) = -10.1               'CG
DNA_DuplexH(1, 3) = DNA_DuplexH(0, 2)   'CT
DNA_DuplexH(2, 0) = -7.7                'GA or TC
DNA_DuplexH(2, 1) = -11.1               'GC
DNA_DuplexH(2, 2) = DNA_DuplexH(1, 1)   'GG
DNA_DuplexH(2, 3) = DNA_DuplexH(0, 1)   'GT
DNA_DuplexH(3, 0) = -6.3                'TA
DNA_DuplexH(3, 1) = DNA_DuplexH(2, 0)   'TC
DNA_DuplexH(3, 2) = DNA_DuplexH(1, 0)   'TG
DNA_DuplexH(3, 3) = DNA_DuplexH(0, 0)   'TT

DNA_EndTAH = 0.4

DNA_DuplexS(0, 0) = -23.6               'AA or TT
DNA_DuplexS(0, 1) = -23                 'AC or GT
DNA_DuplexS(0, 2) = -16.1               'AG or CT
DNA_DuplexS(0, 3) = -18.8               'AT
DNA_DuplexS(1, 0) = -19.3               'CA or TG
DNA_DuplexS(1, 1) = -15.6               'CC or GG
DNA_DuplexS(1, 2) = -25.5               'CG
DNA_DuplexS(1, 3) = DNA_DuplexS(0, 2)   'CT
DNA_DuplexS(2, 0) = -20.3               'GA or TC
DNA_DuplexS(2, 1) = -28.4               'GC
DNA_DuplexS(2, 2) = DNA_DuplexS(1, 1)   'GG
DNA_DuplexS(2, 3) = DNA_DuplexS(0, 1)   'GT
DNA_DuplexS(3, 0) = -18.5               'TA
DNA_DuplexS(3, 1) = DNA_DuplexS(2, 0)   'TC
DNA_DuplexS(3, 2) = DNA_DuplexS(1, 0)   'TG
DNA_DuplexS(3, 3) = DNA_DuplexS(0, 0)   'TT

DNA_InitGCS = -5.9
DNA_InitATS = -9
DNA_SelfS = -1.4

'Initialize the DNA/RNA duplex parameters.
'Parameters found in Sugimoto et al., Biochemistry,
'v. 34, pp. 11,211-11,216 (1995).
'
'Note carefully, these numbers are given for the sequence of
'the DNA strand i.e. we assume that the probe is DNA, the target
'is RNA, and the sequence of the probe is given to the routine.
'For example, the parameter in (0,0) is for dAA/rTT.

DR_DuplexH(0, 0) = -11.5                'AA
DR_DuplexH(0, 1) = -7.8                 'AC
DR_DuplexH(0, 2) = -7#                  'AG
DR_DuplexH(0, 3) = -8.3                 'AU
DR_DuplexH(1, 0) = -10.4                'CA
DR_DuplexH(1, 1) = -12.8                'CC
DR_DuplexH(1, 2) = -16.3                'CG
DR_DuplexH(1, 3) = -9.1                 'CU
DR_DuplexH(2, 0) = -8.6                 'GA
```

```
DR_DuplexH(2, 1) = -8#                  'GC
DR_DuplexH(2, 2) = -9.3                 'GG
DR_DuplexH(2, 3) = -5.9                 'GU
DR_DuplexH(3, 0) = -7.8                 'UA
DR_DuplexH(3, 1) = -5.5                 'UC
DR_DuplexH(3, 2) = -9#                  'UG
DR_DuplexH(3, 3) = -7.8                 'UU

DR_InitH = 1.9

DR_DuplexS(0, 0) = -36.4                'AA
DR_DuplexS(0, 1) = -21.6                'AC
DR_DuplexS(0, 2) = -19.7                'AG
DR_DuplexS(0, 3) = -23.9                'AU
DR_DuplexS(1, 0) = -28.4                'CA
DR_DuplexS(1, 1) = -31.9                'CC
DR_DuplexS(1, 2) = -47.1                'CG
DR_DuplexS(1, 3) = -23.5                'CU
DR_DuplexS(2, 0) = -22.9                'GA
DR_DuplexS(2, 1) = -17.1                'GC
DR_DuplexS(2, 2) = -23.2                'GG
DR_DuplexS(2, 3) = -12.3                'GU
DR_DuplexS(3, 0) = -23.2                'UA
DR_DuplexS(3, 1) = -13.5                'UC
DR_DuplexS(3, 2) = -26.1                'UG
DR_DuplexS(3, 3) = -21.9                'UU

DR_InitS = -3.9

'Load the Zuker hairpin parameters.
FileName = App.Path & "\stack.datd.pw"
ReadZuker FileName, "Stack", DNAHP_StackG, foo
FileName = App.Path & "\stack.dhd.pw"
ReadZuker FileName, "Stack", DNAHP_StackH, foo
CalcSFromGH 4, DNAHP_StackG, DNAHP_StackH, 37#, DNAHP_StackS

FileName = App.Path & "\tstackh.datd.pw"
ReadZuker FileName, "Stack", DNAHP_TStackG, foo
FileName = App.Path & "\tstackh.dhd.pw"
ReadZuker FileName, "Stack", DNAHP_TStackH, foo
CalcSFromGH 4, DNAHP_TStackG, DNAHP_TStackH, 37#, DNAHP_TStackS

FileName = App.Path & "\dangle.datd.pw"
ReadZuker FileName, "Dangle", DNAHP_DangleG, foo
FileName = App.Path & "\dangle.dhd.pw"
ReadZuker FileName, "Dangle", DNAHP_DangleH, foo
CalcSFromGH 4, DNAHP_DangleG, DNAHP_DangleH, 37#, DNAHP_DangleS

FileName = App.Path & "\loop.datd.pw"
ReadZuker FileName, "Loop", DNAHP_LoopG, ZLoopLengths
FileName = App.Path & "\loop.dhd.pw"
ReadZuker FileName, "Loop", DNAHP_LoopH, ZLoopLengths
CalcSFromGH 2, DNAHP_LoopG, DNAHP_LoopH, 37#, DNAHP_LoopS

FileName = App.Path & "\tloop.datd.pw"
ReadZuker FileName, "TetraLoop", DNAHP_TLoopG, ZTetraLoops
FileName = App.Path & "\tloop.dhd.pw"
ReadZuker FileName, "TetraLoop", DNAHP_TLoopH, ZTetraLoops
```



```
CalcSFromGH 1, DNAHP_TLoopG, DNAHP_TLoopH, 37#, DNAHP_TLoopS

FileName = App.Path & "\stack.dat.pw"
ReadZuker FileName, "Stack", RNAHP_StackG, foo
FileName = App.Path & "\stack.dh.pw"
ReadZuker FileName, "Stack", RNAHP_StackH, foo
CalcSFromGH 4, RNAHP_StackG, RNAHP_StackH, 37#, RNAHP_StackS

FileName = App.Path & "\tstackh.dat.pw"
ReadZuker FileName, "Stack", RNAHP_TStackG, foo
FileName = App.Path & "\tstackh.dh.pw"
ReadZuker FileName, "Stack", RNAHP_TStackH, foo
CalcSFromGH 4, RNAHP_TStackG, RNAHP_TStackH, 37#, RNAHP_TStackS

FileName = App.Path & "\dangle.dat.pw"
ReadZuker FileName, "Dangle", RNAHP_DangleG, foo
FileName = App.Path & "\dangle.dh.pw"
ReadZuker FileName, "Dangle", RNAHP_DangleH, foo
CalcSFromGH 4, RNAHP_DangleG, RNAHP_DangleH, 37#, RNAHP_DangleS

FileName = App.Path & "\loop.dat.pw"
ReadZuker FileName, "Loop", RNAHP_LoopG, ZLoopLengths
FileName = App.Path & "\loop.dh.pw"
ReadZuker FileName, "Loop", RNAHP_LoopH, ZLoopLengths
CalcSFromGH 2, RNAHP_LoopG, RNAHP_LoopH, 37#, RNAHP_LoopS

FileName = App.Path & "\tloop.dat.pw"
ReadZuker FileName, "TetraLoop", RNAHP_TLoopG, ZTetraLoops
FileName = App.Path & "\tloop.dh.pw"
ReadZuker FileName, "TetraLoop", RNAHP_TLoopH, ZTetraLoops
CalcSFromGH 1, RNAHP_TLoopG, RNAHP_TLoopH, 37#, RNAHP_TLoopS

End Sub


Private Sub ReadZuker(FileName$, ZType$, Param#(), FirstCol&())
'-------------------------------------------------------------------------------
'Function
'   Read thermodynamic parameters for structure calculations.
'Arguments
'   Filename: The data file.
'   ZType: The type of parameter array (see below).
'   Param: The parameter array to be filled in.
'   FirstCol: The values from the first column.
'Notes
'   1. This routine reads data files originally designed to be read by
'      MFOLD; the files must be edited to place a # sign on lines that
'      do not contain data, and to replace all the "." entries by 0.
'   2. There are several types of parameter files.  Type "stack"
'      gives the parameters for stacking one base pair over another,
'      and thus has 256 entries.  Type "dangle" gives the parameters
'      for dangling a base over a pair, and thus has 64 parameters.
'      The other types are particular to the parameters being
'      presented.
'   3. The FirstCol argument is used only by Loop and TetraLoop types.
'   4. In all these files, the order of bases is ACGU->0,1,2,3
'-------------------------------------------------------------------------------
```



```
Dim ZFile&          'parameter file
Dim FileLength&     'length of parameter file
Dim LineLength&     'length of one line
Dim WordLength&     'length of one word
Dim FileStr$        'text of read-in file
Dim LineStr$        'text of one line
Dim WordStr$        'text of one word
Dim A&, B&, X&, Y&, L&, C&, T& 'loop indices
Dim NumSeq%(0 To 3) 'numeric representation of tetraloop sequences

'Open the file, read it in.
ZFile = FreeFile
Open FileName For Binary As #ZFile
FileLength = FileLen(FileName)
FileStr = Input(FileLength, #ZFile)

'Choose how to parse the file.
Select Case ZType

Case "Stack"
'These entries represent stacking of one base pair over another:
'   5'-AX-3'
'   3'-BY-5'.
'In each row, Y varies fast, B varies slow.
'By row, X varies fast, and A varies slowly.
'Index order used is param(A,X,B,Y).
For A = 0 To 3
    For X = 0 To 3
        NextLine FileStr, FileLength, LineStr, LineLength, "#"
        For B = 0 To 3
            For Y = 0 To 3
                NextWord LineStr, LineLength, WordStr, WordLength
                Param(A, X, B, Y) = CDbl(WordStr)
            Next Y
        Next B
    Next X
Next A

Case "Dangle"
'These entries represent dangles of 3' ends, then the 5' ends:
'   5'-AX-3'
'   3'-B -5' is a 3' dangle
'and
'   5'-A -3'
'   3'-BY-5' is a 5' dangle.
'In each row, X or Y varies fast, and B varies slowly.
'By row, A varies.
'Index order used is param(0,A,X,B) for 3', and param(1,A,B,Y) for the 5'
dangles.
For A = 0 To 3
    NextLine FileStr, FileLength, LineStr, LineLength, "#"
    For B = 0 To 3
        For X = 0 To 3
            NextWord LineStr, LineLength, WordStr, WordLength
            Param(0, A, X, B) = CDbl(WordStr)
        Next X
    Next B
```



```
Next A
For A = 0 To 3
    NextLine FileStr, FileLength, LineStr, LineLength, "#"
    For B = 0 To 3
        For Y = 0 To 3
            NextWord LineStr, LineLength, WordStr, WordLength
            Param(1, A, B, Y) = CDbl(WordStr)
        Next Y
    Next B
Next A

Case "Loop"
'These entries represent internal=0, bulge=1, and hairpin=2 loops.
'By row, looplength L varies.
'Index order used is param(looptype,L)
'FirstCol is filled from the first column, representing loop lengths.
For L = 0 To NumZukerLoops - 1
    NextLine FileStr, FileLength, LineStr, LineLength, "#"
    NextWord LineStr, LineLength, WordStr, WordLength
    FirstCol(L) = CLng(WordStr)
    For T = 0 To 2
        NextWord LineStr, LineLength, WordStr, WordLength
        Param(T, L) = CDbl(WordStr)
    Next T
Next L

Case "TetraLoop"
'These represent especially stable tetraloops.
'By row, sequence of the tetraloop varies.
'Firstcol is filled with a quaternary index of the sequence.
For L = 0 To NumTetraLoops - 1
    NextLine FileStr, FileLength, LineStr, LineLength, "#"
    NextWord LineStr, LineLength, WordStr, WordLength
    DNA_Str2Num WordStr, NumSeq
    FirstCol(L) = 0
    For C = 0 To 3
        FirstCol(L) = FirstCol(L) * 4 + NumSeq(C)
    Next
    NextWord LineStr, LineLength, WordStr, WordLength
    Param(L) = CDbl(WordStr)
Next L

End Select
Close ZFile
End Sub

Function DR_CalcDeltaH#(ByVal Seq$)
'-------------------------------------------------------------------------------
'Function
'   Calculate the association enthalpy for a given RNA sequence
'   with its perfect W-C DNA complement.
'Arguments
'   Seq: The RNA sequence.
'Returns
'   Enthalpy of association, in kcal/mole.
'Notes
'   1. Standard conditions (1M NA+) is assumed.
'History
```

```
'   29-Jul-1997: From PkW's routine of the same name.  PW.
'-------------------------------------------------------------------------

Dim Length&          'length of the sequence
Dim B&               'base index for traversing the sequence
Dim NumSeq%()        'numerical representation of the sequence

'Lower case, please.
Seq = LCase(Seq)

'Create the numeric representation.
Length = Len(Seq)
ReDim NumSeq(0 To Length - 1)
DNA_Str2Num Seq, NumSeq

'Initialize with initiation deltaH
DR_CalcDeltaH = DR_InitH

'Sum the nearest neighbor values along the strand.
For B = 1 To Length - 1
    DR_CalcDeltaH = DR_CalcDeltaH + DR_DuplexH(NumSeq(B - 1), NumSeq(B))
Next B

'Convert to cal.
DR_CalcDeltaH = DR_CalcDeltaH * 1000#

End Function


Function DNA_CalcDeltaH#(ByVal Seq$)
'-------------------------------------------------------------------------
'Function
'   Calculate the association enthalpy for a DNA sequence
'   with its perfect W-C complement.
'Arguments
'   Seq: The sequence.
'Returns
'   Enthalpy of association, in kcal/mole.
'Notes
'   1. Standard conditions (1M NA+) is assumed.
'History
'   29-Jul-1997: From PkW's routine of the same name.  PW.
'-------------------------------------------------------------------------

Dim Length&          'length of the sequence
Dim B&               'base index for traversing the sequence
Dim NumSeq%()        'numerical representation of the sequence

'Lower case, please.
Seq = LCase(Seq)

'Create the numeric representation.
Length = Len(Seq)
ReDim NumSeq(0 To Length - 1)
DNA_Str2Num Seq, NumSeq

'Initialize with AT end correction.
If NumSeq(0) = 3 Then DNA_CalcDeltaH = DNA_CalcDeltaH + DNA_EndTAH
```



```
If NumSeq(Length - 1) = 0 Then DNA_CalcDeltaH = DNA_CalcDeltaH + DNA_EndTAH

'Sum the nearest neighbor values along the strand.
For B = 1 To Length - 1
    DNA_CalcDeltaH = DNA_CalcDeltaH + DNA_DuplexH(NumSeq(B - 1), NumSeq(B))
Next B

'Convert to cal.
DNA_CalcDeltaH = DNA_CalcDeltaH * 1000#

End Function


Function DNA_CalcDeltaS#(ByVal Seq$)
'-------------------------------------------------------------------------
'Function
'   Calculate the association entropy for a sequence
'   with its perfect W-C complement.
'Arguments
'   Seq: The sequence
'Returns:
'   Entropy of association, in cal/mole/deg K.
'Notes
'   1. Parameters are derived from SantaLucia et al., Biochemistry,
'      v. 35, pp. 3555-3562 (1996).
'   2. Standard conditions (1M NA+) is assumed.
'History
'   29-Jul-1997: From PkW's routine of the same name.  PW.
'-------------------------------------------------------------------------

Dim Length&          'length of the sequence
Dim B&               'base index for traversing the sequence
Dim NumSeq%()        'numerical representation of the sequence

'Lower case, please.
Seq = LCase(Seq)

'Create the numeric representation.
Length = Len(Seq)
ReDim NumSeq(Length - 1)
DNA_Str2Num Seq, NumSeq

'Initialize with self-symmetry correction.
If Seq = DNA_RevComp(Seq) Then DNA_CalcDeltaS = DNA_CalcDeltaS + DNA_SelfS

'add initiation term
If InStr(Seq, "c") Or InStr(Seq, "g") Then
    DNA_CalcDeltaS = DNA_CalcDeltaS + DNA_InitGCS
Else
    DNA_CalcDeltaS = DNA_CalcDeltaS + DNA_InitATS
End If

'Sum the nearest neighbor values along the strand
For B = 1 To Length - 1
    DNA_CalcDeltaS = DNA_CalcDeltaS + DNA_DuplexS(NumSeq(B - 1), NumSeq(B))
Next B
```

```
End Function


Function DR_CalcDeltaS#(ByVal Seq$)
' ------------------------------------------------------------------------
'Function
'   Calculate the association entropy for a given RNA sequence
'   with its perfect W-C DNA complement.
'Arguments
'   Seq: The sequence
'Returns:
'   Entropy of association, in cal/mole/deg K.
'Notes
'   1. Standard conditions (1M NA+) is assumed.
'History
'   29-Jul-1997: From PkW's routine of the same name.  PW.
' ------------------------------------------------------------------------

Dim Length&          'length of the sequence
Dim B&               'base index for traversing the sequence
Dim NumSeq%()        'numerical representation of the sequence

'Lower case, please.
Seq = LCase(Seq)

'Create the numeric representation.
Length = Len(Seq)
ReDim NumSeq(0 To Length - 1)
DNA_Str2Num Seq, NumSeq

'Begin with initiation term.
DR_CalcDeltaS = DR_InitS

'Sum the nearest neighbor values along the strand.
For B = 1 To Length - 1
    DR_CalcDeltaS = DR_CalcDeltaS + DR_DuplexS(NumSeq(B - 1), NumSeq(B))
Next B

End Function

Public Sub DNA_CalcAllTM(Seq$(), tmp As cTMPars, TM#())
'------------------------------------------------------------------------
'Function
'   Calculate the melting temperature of an array of oligos with their
'   perfect W-C complements.
'Arguments
'   Seq: The sequences
'   TMP: An instance of the parameter class for TM calculations.
'Returns
'   Melting temperature, in deg. C.
'Notes
'   1. Concentration is used as is, i.e. assuming the complement
'   is present at much lower concentration.
'History
'   29-Jul-1997: From PkW's routine of the same name.  PW.
' ------------------------------------------------------------------------
```



```
On Error GoTo E

Dim NumSeqs&          'number of sequences we are working with
Dim S&                'index

'Determine the number of probes we are calculating TM for.
NumSeqs = UBound(Seq) + 1

'Calculate melting points
For S = 0 To NumSeqs - 1
    If S - Progress.StopAt = 0 Then Progress.CheckProgress S
    TM(S) = DNA_CalcTM(Seq(S), tmp.Conc)
Next S
Exit Sub

E: Debug.Print "Error in DNA_CalcAllTM"
Err.Raise Err.Number, , Err.Description
End Sub

Public Sub DNA_CalcClamp(Seq$(), TClamp As cClampPars, Clamp#())
'------------------------------------------------------------------------
'Function
'   Calculate the melting temperature of the Clamp of a probe
'   to its perfect W-C complement.
'Arguments
'   Seq: The sequences
'   TClamp: An instance of the parameter class for Clamp calculations.
'Returns
'   Melting temperature of tightest clamp, in deg. C.
'Notes
'   1. Concentration is used as is, i.e. assuming the complement
'   is present at much lower concentration.
' ------------------------------------------------------------------------
On Error GoTo E

Dim NumSeqs&          'number of sequences we are working with
Dim S&, SS&           'indices
Dim SubSeq$           'subsequence
Dim BestTM#, ThisTM#  'current most stable clamp

'Determine the number of probes we are calculating Clamp for.
NumSeqs = UBound(Seq) + 1

'Calculate melting points.
For S = 0 To NumSeqs - 1
    If S - Progress.StopAt = 0 Then Progress.CheckProgress S
    BestTM = 0
    For SS = TClamp.FiveP + 1 To Len(Seq(S)) - TClamp.ThreeP - TClamp.Length + 1
        SubSeq = Mid(Seq(S), SS, TClamp.Length)
        ThisTM = DNA_CalcTM(SubSeq, TClamp.Conc) + 273.15
        If ThisTM > BestTM Then BestTM = ThisTM
    Next SS
    Clamp(S) = BestTM
Next S
Exit Sub
E: Debug.Print "Error in DNA_CalcClamp"
Err.Raise Err.Number, , Err.Description
End Sub
```



```
Public Sub DR_CalcClamp(Seq$(), TClamp As cClampPars, Clamp#())
'-------------------------------------------------------------------------------
'Function
'   Calculate the melting temperature of the Clamp of a probe
'   to its perfect RNA W-C complement.
'Arguments
'   Seq: The sequences
'   TClamp: An instance of the parameter class for Clamp calculations.
'Returns
'   Melting temperature of tightest clamp, in deg. C.
'Notes
'   1. Concentration is used as is, i.e. assuming the complement
'   is present at much lower concentration.
' ------------------------------------------------------------------------------
On Error GoTo E

Dim NumSeqs&          'number of sequences we are working with
Dim S&, SS&           'indices
Dim SubSeq$           'subsequence
Dim BestTM#, ThisTM#  'current most stable clamp

'Determine the number of probes we are calculating Clamp for.
NumSeqs = UBound(Seq) + 1

'Calculate melting points.
For S = 0 To NumSeqs - 1
    If S - Progress.StopAt = 0 Then Progress.CheckProgress S
    BestTM = 0
    For SS = TClamp.FiveP + 1 To Len(Seq(S)) - TClamp.ThreeP - TClamp.Length + 1
        SubSeq = Mid(Seq, SS, TClamp.Length)
        ThisTM = DR_CalcTM(SubSeq, TClamp.Conc) + 273.15
        If ThisTM > BestTM Then BestTM = ThisTM
    Next SS
    Clamp(S) = BestTM
Next S
Exit Sub
E: Debug.Print "Error in DR_CalcClamp"
Err.Raise Err.Number, , Err.Description
End Sub

Public Sub DNA_CalcdGH(Seq$(), dGHP As cDGHPars, dGH#())
'-------------------------------------------------------------------------------
'Function
'   Calculate the hairpin dGs of an array of oligos.
'Arguments
'   Seq: The sequences.
'   dGHP: An instance of the parameter class for dGH calculations.
'   dGH: The hairpin dGs.
' ------------------------------------------------------------------------------
On Error GoTo E

Dim NumSeq%()    'numeric representation of the sequence
Dim S&           'index

'Recalculate all G parameter matrices at current temperature.
```



```
CalcGFromHS 4, DNAHP_StackH, DNAHP_StackS, dGHP.T, DNAHP_StackG
CalcGFromHS 4, DNAHP_TStackH, DNAHP_TStackS, dGHP.T, DNAHP_TStackG
CalcGFromHS 4, DNAHP_DangleH, DNAHP_DangleS, dGHP.T, DNAHP_DangleG
CalcGFromHS 2, DNAHP_LoopH, DNAHP_LoopS, dGHP.T, DNAHP_LoopG
CalcGFromHS 1, DNAHP_TLoopH, DNAHP_TLoopS, dGHP.T, DNAHP_TLoopG

'Calculate dGs.
For S = 0 To UBound(Seq)
    If S - Progress.StopAt = 0 Then Progress.CheckProgress S
    ReDim NumSeq(0 To Len(Seq(S)) - 1)
    DNA_Str2Num Seq(S), NumSeq
    dGH(S) = DNA_BestHairpin(NumSeq)
Next S
Exit Sub
E: Debug.Print "Error in DNA_CalcGH"
Err.Raise Err.Number, , Err.Description
End Sub

Public Sub DNA_CalcdGD(Seq$(), dGDP As cdGDPars, dGD#())
'-------------------------------------------------------------------------------
'Function
'   Calculate the duplex dGs of an array of oligos.
'Arguments
'   Seq: The sequences.
'   dGDP: An instance of the parameter class for dGD calculations.
'   dGD: The hairpin dGs.
' ------------------------------------------------------------------------------
On Error GoTo E

Dim NumSeq%()    'numeric representation of the sequence
Dim S&           .'index

'Calculate dGs.
For S = 0 To UBound(Seq)
    If S - Progress.StopAt = 0 Then Progress.CheckProgress S
    dGD(S) = (DNA_CalcDeltaH(Seq(S)) - (dGDP.T + 273.15) *
DNA_CalcDeltaS(Seq(S))) / 1000#
Next S
Exit Sub
E: Debug.Print "Error in DNA_CalcGD"
Err.Raise Err.Number, , Err.Description
End Sub

Public Sub DR_CalcdGD(Seq$(), dGDP As cdGDPars, dGD#())
'-------------------------------------------------------------------------------
'Function
'   Calculate the duplex dGs of an array of oligos.
'Arguments
'   Seq: The sequences.
'   dGDP: An instance of the parameter class for dGD calculations.
'   dGD: The hairpin dGs.
' ------------------------------------------------------------------------------
On Error GoTo E

Dim NumSeq%()    'numeric representation of the sequence
Dim S&           'inex

'Calculate dGs.
```

```
For S = 0 To UBound(Seq)
    If S - Progress.StopAt = 0 Then Progress.CheckProgress S
    dGD(S) = (DR_CalcDeltaH(Seq(S)) - (dGDP.T + 273.15) * DR_CalcDeltaS(Seq(S)))
/ 1000#
Next S
Exit Sub
E: Debug.Print "Error in DNA_CalcGD"
Err.Raise Err.Number, , Err.Description
End Sub


Public Sub DNA_CalcdGM(Seq$(), dGMP As cdGMPars, dGM#())
'-------------------------------------------------------------------------
'Function
'   Calculate the MFold dGs of an array of oligos.
'Arguments
'   Seq: The sequences.
'   dGMP: An instance of the parameter class for dGM calculations.
'   dGM: The hairpin dGs.
' -------------------------------------------------------------------------
On Error GoTo E

Dim S&          'index

'Calculate dGs.
For S = 0 To UBound(Seq)
    If S - Progress.StopAt = 0 Then Progress.CheckProgress S
    dGM(S) = frmMain.Mfoldx.mfold(Seq(S), Val(dGMP.T), True)
Next S
Exit Sub

E: Debug.Print "Error in DNA_CalcdGM."
Err.Raise Err.Number, , Err.Description
End Sub


Public Sub RNA_CalcdGH(Seq$(), dGHP As cDGHPars, dGH#())
'-------------------------------------------------------------------------
'Function
'   Calculate the hairpin dGs of an array of oligos.
'Arguments
'   Seq: The sequences.
'   dGHP: An instance of the parameter class for dGH calculations.
'   dGH: The hairpin dGs.
' -------------------------------------------------------------------------
On Error GoTo E

Dim NumSeq%()   'numeric representation of the sequence
Dim S&          'index

'Recalculate all G parameter matrices at current temperature.
CalcGFromHS 4, RNAHP_StackH, RNAHP_StackS, dGHP.T, RNAHP_StackG
CalcGFromHS 4, RNAHP_TStackH, RNAHP_TStackS, dGHP.T, RNAHP_TStackG
CalcGFromHS 4, RNAHP_DangleH, RNAHP_DangleS, dGHP.T, RNAHP_DangleG
CalcGFromHS 2, RNAHP_LoopH, RNAHP_LoopS, dGHP.T, RNAHP_LoopG
CalcGFromHS 1, RNAHP_TLoopH, RNAHP_TLoopS, dGHP.T, RNAHP_TLoopG

'Calculate dGs.
```



```
For S = 0 To UBound(Seq)
    If S - Progress.StopAt = 0 Then Progress.CheckProgress S
    ReDim NumSeq(0 To Len(Seq(S)) - 1)
    DNA_Str2Num Seq(S), NumSeq
    dGH(S) = RNA_BestHairpin(NumSeq)
Next S
Exit Sub
E: Debug.Print "Error in RNA_CalcdGH"
Err.Raise Err.Number, , Err.Description
End Sub

Public Sub RNA_CalcdGM(Seq$(), dGMP As cdGMPars, dGM#())
'-------------------------------------------------------------------------
'Function
'   Calculate the MFold dGs of an array of oligos.
'Arguments
'   Seq: The sequences.
'   dGMP: An instance of the parameter class for dGM calculations.
'   dGM: The MFold dGs.
' -------------------------------------------------------------------------
On Error GoTo E

Dim S&          'index

'Calculate dGs.
For S = 0 To UBound(Seq)
    If S - Progress.StopAt = 0 Then Progress.CheckProgress S
    dGM(S) = frmMain.Mfoldx.mfold(Seq(S), Val(dGMP.T), False)
Next S
Exit Sub
E: Debug.Print "Error in RNA_CalcGM"  .
Err.Raise Err.Number, , Err.Description
End Sub


Public Sub DR_CalcAllTM(Seq$(), tmp As cTMPars, TM#())
'-------------------------------------------------------------------------
'Function
'   Calculate the melting temperature of an array of DNA probes with their
'   perfect W-C RNA target complements.
'Arguments
'   Seq: The sequences.
'   TMP: An instance of the parameter class for TM calculations.
'Returns
'   Melting temperature, in deg. C.
'Notes
'   1. Concentration is used as is, i.e. assuming the complement
'   is present at much lower concentration.
'History
'   29-Jul-1997: From PkW's routine of the same name.  PW.
' -------------------------------------------------------------------------
On Error GoTo E
Dim NumSeqs&            'number of sequences we are working with
Dim S&                  'index

'Determine the number of probes we are calculating TM for.
NumSeqs = UBound(Seq) - LBound(Seq) + 1
```



```
'Calculate melting points
For S = 0 To NumSeqs - 1
    If S - Progress.StopAt = 0 Then Progress.CheckProgress S
    TM(S) = DR_CalcTM(Seq(S), tmp.Conc)
Next S
Exit Sub
E: Debug.Print "Error in DR_CalcAllTM"
Err.Raise Err.Number, , Err.Description
End Sub

Public Function DR_CalcTM#(Seq$, Conc#)
'--------------------------------------------------------------------------
'Function
'   Calculate the TM of one DNA/RNA duplex.  Sequence given is DNA.
'--------------------------------------------------------------------------
DR_CalcTM = DR_CalcDeltaH(Seq) / (DR_CalcDeltaS(Seq) + RGas * Log(Conc)) -
273.15
End Function

Public Function DNA_CalcTM#(Seq$, Conc#)
'--------------------------------------------------------------------------
'Function
'   Calculate the TM of one DNA/DNA duplex.
'--------------------------------------------------------------------------
DNA_CalcTM = DNA_CalcDeltaH(Seq) / (DNA_CalcDeltaS(Seq) + RGas * Log(Conc)) -
273.15
End Function
```

```
Attribute VB_Name = "Utilities"
Option Explicit

'A quicksort routine for doubles, based on NR code.
Private Declare Function vb5sort2 Lib "vb5nrdll.dll" _
    (ByVal N&, ByRef Vector#, ByRef Index&) As Long

Public Sub CalcRun(Pos&(), RP As cRunPars, Run&())
'--------------------------------------------------------------------------
'Function
'   Calculate the runs in a set of positions.
'Arguments
'   Pos: The positions.
'   RP: The run parameters.
'   Run: The returned run.
'Method
'   Begin by looping over all positions, extending down over all consecutive
'   entries, then up over all consecutive entries.  Then mark in the active
'   position the length of run found.  Next, eliminate all runs that are
'   too short.  Finally, for each run, mark the requested number of members
'   of the run, at the requested spacing, as being members.  If the run is
'   shorter than can accomodate the requested number of members, fill in as
'   many as possible, while still preserving the requested spacing.
'Notes
'   1. Run must be allocated and sized correctly by the caller.
'--------------------------------------------------------------------------
Dim P&              'index
Dim EndUp&          'end of the run, counting up
Dim EndDown&        'end of the run, counting down
```



```
Dim StepDir&        'current step direction
Dim M&              'index

'Loop over positions.
For P = 0 To UBound(Pos)
    EndDown = P
    Do While ((P - EndDown) = (Pos(P) - Pos(EndDown)))
        EndDown = EndDown - 1
        If EndDown = -1 Then Exit Do
    Loop
    EndDown = EndDown + 1
    EndUp = P
    Do While ((EndUp - P) = (Pos(EndUp) - Pos(P)))
        EndUp = EndUp + 1
        If EndUp = UBound(Pos) + 1 Then Exit Do
    Loop
    EndUp = EndUp - 1
    Run(P) = EndUp - EndDown + 1
Next P

'Remove runs that are too short.
For P = 0 To UBound(Run)
    If Run(P) < RP.Min Then Run(P) = 0
Next P

'Pick out requested elements from each run.
P = 0
Do While P < UBound(Run)

    'Find next run.
    Do While Run(P) = 0 And P < UBound(Run)
        P = P + 1
    Loop

    'Record the ends, step to the middle (to the right of middle for even
lengths).
    EndDown = P
    EndUp = P + Run(P) - 1
    P = Fix((EndDown + EndUp + 1) / 2)

    'Mark elements, stepping down from the middle first.  This ensures that all
    'elements get marked for even lengths, spacing=1.
    StepDir = -1
    For M = 1 To RP.Num
        Run(P) = -Run(P)
        P = P + StepDir * M * RP.Spacing
        If (P < EndDown Or P > EndUp) Then Exit For
        StepDir = StepDir * -1
    Next M

    'Move over this run.
    P = EndUp + 1
Loop

'Convert marked elements back to run length.
For P = 0 To UBound(Run)
    If Run(P) > 0 Then Run(P) = 0
    If Run(P) < 0 Then Run(P) = -Run(P)
```

```
Next P

End Sub

Public Function IsGoodRS(RS As Recordset) As Boolean
'--------------------------------------------------------------------------
'Function
'   Check whether it is possible to access the records of
'   recordset connected to the sequence, probeset, or probes table.
'   No current record need exist for this function to return True.
'--------------------------------------------------------------------------
On Error GoTo Err
IsGoodRS = False
If IsNull(RS) Then Exit Function
If (RS.EOF = True And RS.BOF = True) Then Exit Function
IsGoodRS = True
Err:
End Function

Public Function NumRecords&(RS As Recordset)
'--------------------------------------------------------------------------
'Function
'   Count the number of records in the recordset.
'Notes
'   A side effect of this routine is to position the recordset at the
'   first record.
'--------------------------------------------------------------------------
RS.MoveLast
RS.MoveFirst
NumRecords = RS.RecordCount
End Function

Public Sub UnPackSequence(ByRef SeqStr As String, ByRef NumStr As String)
'SeqStr is assumed to hold a packed sequence.
Dim NewSeqStr$
Dim Bases&, I&

NumStr = ""

Bases = 1
Do While Len(SeqStr) - Bases > 60
    NumStr = NumStr & Format(Format(Bases, "#####"), "@@@@@") & vbCrLf
    For I = Bases To Bases + 59
        NewSeqStr = NewSeqStr & Mid(SeqStr, I, 1)
        If (I Mod 10 = 0) Then NewSeqStr = NewSeqStr & " "
    Next I
    Bases = Bases + 60
    NewSeqStr = NewSeqStr + vbCrLf
Loop
If Bases <> Len(SeqStr) Then
    NumStr = NumStr & Format(Format(Bases, "#####"), "@@@@@")
    For I = Bases To Len(SeqStr)
        NewSeqStr = NewSeqStr & Mid(SeqStr, I, 1)
        If (I Mod 10 = 0) Then NewSeqStr = NewSeqStr & " "
    Next I
End If
SeqStr = NewSeqStr
End Sub
```



```
Public Function StrField$(Str$, lineLen&, ByVal FieldLen&, Optional Extra&)
'Returns a leading field of a string, and shortens the string
'by the fieldlength + extra.
'If the line is too short, as much of the requested field as possible
'will be returned.

If FieldLen > lineLen Then FieldLen = lineLen
StrField = Trim(Left(Str, FieldLen))
lineLen = lineLen - FieldLen
If Not IsMissing(Extra) Then
    lineLen = lineLen - Extra
    If lineLen < 0 Then lineLen = 0
End If
Str = Right(Str, lineLen)
End Function

Public Sub NextWord(Str$, StrLen&, Word$, WordLen&)
'Strips leading spaces, copies leading word, reduces linelength
Do While ((Mid(Str, 1, 1) = " ") And (StrLen > 0))
    StrLen = StrLen - 1
    Str = Right(Str, StrLen)
Loop
If InStr(Str, " ") <> 0 Then
    WordLen = InStr(Str, " ") - 1
Else
    WordLen = StrLen
End If
If WordLen <> 0 Then
    Word = Mid(Str, 1, WordLen)
    StrLen = StrLen - WordLen
    Str = Right(Str, StrLen)
Else
    Word = ""
    Str = ""
    StrLen = 0
    WordLen = 0
End If
End Sub

Public Sub NextLine(FileStr$, FileLen&, LineStr$, lineLen&, CommentChar$)
'Return the next line that doesn't begin with CommentChar
lineLen = 0
Do While (lineLen = 0 And FileLen > 0)
    lineLen = InStr(FileStr, vbCrLf) - 1
    LineStr = Left(FileStr, lineLen)
    FileLen = FileLen - lineLen - 2
    FileStr = Right(FileStr, FileLen)
    If (Mid$(LineStr, 1, 1) = CommentChar) Then lineLen = 0
Loop
End Sub

Public Sub QuickSort(X#(), Index&(), LB&, UB&)
'Quicksorts the array X into ascending order,
'simultaneously sorting I to provide a sort index.
Dim N&, foo&
N = UB - LB + 1
foo = vb5sort2(N, X(LB), Index(LB))
```

```
End Sub

Public Function BinarySearch&(Vector#(), Value#, U&, L&)
'-------------------------------------------------------------------------
'Function
'   Find the index of a value in a sorted array.
'-------------------------------------------------------------------------
Do While U <> L
    BinarySearch = (U + L) / 2
    If Vector(BinarySearch) = Value Then Exit Function
    If Vector(BinarySearch) > Value Then
        U = BinarySearch
    Else
        L = BinarySearch
    End If
Loop
End Function

Public Function PackSequence$(SeqStr$)
Dim NewStr$, Ch$
Dim I&
For I = 1 To Len(SeqStr)
    Ch = Mid$(SeqStr, I, 1)
    If InStr("ACGTUacgtu", Ch) Then PackSequence = PackSequence & Ch
Next I
End Function
```

